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CHROMOSOME NUMBER AND BEHAVIOUR IN A PLANT BREEDER'S SAMPLE OF PENTAPLOID WHEAT HYBRID DERIVATIVES[1]

BY R. MERTON LOVE[2]

Abstract

Meiosis was studied in *Triticum vulgare* vars. Marquis, Marquillo, Hope, and R.L. 729, in *T. durum* var. Iumillo, and in 336 rust resistant selections from their pentaploid hybrids. R.L. 729 contains some plants that are unstable cytologically. In the fifth to seventh generation material, in fifty lines that had been subjected to rigorous selection, plants were found with 28, 38, 39, 40, 41, 42, and 43 chromosomes. Less than 42% of the plants had 42 chromosomes—the Vulgare number.

Fifty per cent of the plants were heterozygous for the arrangement of one or more chromosome segments. The chromosome aberrations detected were, in order of frequency: inversions, translocations, deficiencies, and duplications. All types of aberration were transmitted from F_7 to F_8 in some cases. One chromosome deficient for a complete arm arose from an isochromosome, and one isochromosome arose from a chromosome deficient for a complete arm.

The incidence of natural crossing in the aberrant plants is much higher than in normal wheats.

It is suggested (1) that plant breeders use as parents only those plants known to be stable cytologically; (2) that a more intensive cytological analysis of hybrid derivatives would be invaluable to the plant breeder; and (3) that the plant breeder should not overlook hybrid derivatives with specific chromosome rearrangements since such plants may be the means of breaking the linkage between "desirable" and "undesirable" genes.

Introduction

A strain of *Triticum durum* var. Iumillo ($2n = 28$) was crossed with four varieties of *Triticum vulgare* ($2n = 42$) in an attempt to transfer the full immunity to stem rust possessed by Iumillo to the Vulgare wheats. Fifty of the Vulgare-like, rust-resistant lines derived from these crosses were studied cytologically at Ottawa in 1937. This was one of several studies made on the material in that year. A summary of the cytological results (15) as well as a general outline of those of particular interest to plant breeders (22) have already been published.

Since this is probably the first cytological paper that deals with a plant breeder's sample of derivatives of pentaploid wheat hybrids, it may be of interest to record how the selections were made.

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The crosses were made by Dr. K. W. Neatby at the Dominion Rust Research Laboratory, Winnipeg, Canada, in 1930, 1931, and 1932. Since 1935, Dr. R. F. Peterson of that institution has been in charge of the project.

All hybrid generations were grown under artificially induced stem rust epidemics at the Dominion Rust Research Laboratory. According to Dr. Peterson, the "Vulgare-ness" of the hybrid plants was judged chiefly on spike and glume characters. In the early hybrid generations any plant with a high degree of rust resistance, and with spikes tending toward the Vulgare type, was saved. It was considered that the progeny of these plants would include some true Vulgare types, and that lines possessing the immunity of Iumillo, although not typical Vulgare wheats in other respects, might prove useful as breeding material. Selection for Vulgare-like spike was followed more strictly in each succeeding generation. In 1936 the 50 lines remaining in the test were virtually free from stem rust. Forty-eight of these lines were Vulgare or near-Vulgare in type and two (from Marquillo × Iumillo) were Durum-like but possessed some Vulgare characteristics and were kept because of their marked resistance to stem rust. These two lines were later found to be 28-chromosome wheats.

Material

In Table I are included details of the original crosses, the number of plants examined at first metaphase and at the young pollen tetrad stage, the number of lines in 1937, the number of F_2 plants from which these lines were derived, and the total number of F_2 plants grown.

TABLE I

MATERIAL STUDIED, AND ITS SOURCE

Cross	Generation in 1937	Number of plants studied cytologically in 1937		Number of lines in 1937	Number of F_2 plants from which the lines were derived	Total number of F_2 plants grown
		First division	Young tetrads			
Marquillo × Iumillo	F_5	102	56	13	5	4,900
Iumillo × Hope	F_6, F_7	110	63	17	3	2,400
Marquis × Iumillo	F_7	50	21	9	2	2,600
Iumillo × R.L. 729	F_6, F_7	74	35	11	4	6,000
Totals		336	175	50	14	15,900

Of the four varieties of *T. vulgare* used as parents, Marquis (R.L. 84)* is the only one that is not a derivative of a pentaploid hybrid. Hope (R.L. 209), R.L. 729, and Marquillo (R.L. 1943) are more or less rust-resistant derivatives of the crosses Marquis × Yaroslav Emmer, Pentad Durum × Marquis, and Marquis × Iumillo, respectively.

* *Rust Laboratory Accession Number.*

In 1937, 23 plants of the parent varieties were examined, this number representing from four to six plants in each variety.

In 1938, 47 plants, the progeny of 18 of the hybrid derivatives examined in 1937, were studied cytologically. Seventeen plants were aberrant and one was "normal" cytologically; the 18 plants were not selfed since the aim was to ascertain the diminution or increase, as well as the dispersion, of cytological irregularities under field conditions. The progeny of the normal plant served as a check on those of the remaining 17.

In order to obviate the necessity of repeating the cytological details, the plants used as parents for the 1938 studies are indicated in the "1937 Observations".

Methods

Twenty-five seeds of each of the 50 lines and 5 parent varieties were sown at Ottawa in 1937. The seeds were planted in rows 7 in. apart and spaced 2 in. in the rows. The seeds from the 18 plants grown in 1938 were similarly treated. Spacing of the plants results in greater tillering—an important feature when one or more tillers are taken for cytological purposes.

Anthers at the meiotic stages were fixed overnight in a mixture of absolute ethyl alcohol and glacial acetic acid (3 : 1). Wheat anthers fixed singly smear better and give much clearer division figures than those fixed in the glumes. The next day the anthers were washed in 70% ethyl alcohol and stored in vials of fresh 70% alcohol. The best grade of cork must be used to ensure against discoloration of the anthers if they are to be stored several months.

All cytological studies, except those on young pollen tetrads, were made from permanent iron-aceto-carmine smear slides. The method is a modification of the technique outlined by McClintock (18).

Staining Schedule for Smear Preparations

Only the chief departures from McClintock's smear technique will be mentioned.

(1) After the cover glass has been applied, the slide is heated over a steam bath. Steam has two advantages over a dry flame: there is a more even heat, and there is no danger of boiling the stain and thus drying out the slide.

(2) After pressure has been applied to the cover glass and the slide has been allowed to cool, both are placed in a coplin jar containing a mixture of glacial acetic acid, absolute ethyl alcohol, and xylol (3 : 1 : 1). The cover glass usually drops to the bottom of the jar in two or three minutes.

(3) Then the slide and cover glass are run through the following mixtures in coplin jars. Two or three minutes in each mixture is sufficient.

Glacial acetic acid, absolute alcohol, and xylol (1 : 1 : 1).
Absolute alcohol and xylol (1 : 1).
Absolute alcohol and xylol (1 : 1).

(4) Mount in neutral damar.

From 20 to 150 complete nuclei were analysed in each plant studied at the first meiotic divisions. For the young pollen tetrad studies, 100 pollen mother-cells were examined in a plant and the numbers of micronuclei and inversion bridges were recorded for each.

A Zeiss apo. 1.4 N.A. objective and 7 × ocular were used for photomicrographs × 560 and an apo. 1.3 N.A. for those × 1100.

1937 Observations

A. Parents

Triticum durum var. Iumillo. Each of the four plants examined at the first meiotic divisions had 28 chromosomes which regularly formed 14_{II}. No multivalents were found. In the three plants studied at the young pollen tetrad stage, the percentage of tetrads with one or two micronuclei was 0, 3 and 6. No inversion bridges were detected.

T. vulgare vars. Marquis, Marquillo, and Hope. Four, five, and six plants, respectively, were examined in the three varieties. All plants had 42 chromosomes, although other studies conducted by the author and others (5, 23) have shown aneuploid plants to be not uncommon in Marquillo and Hope. In the nine plants of the three varieties examined at the tetrad stage, the percentage of tetrads with one or two micronuclei varied from 1 to 3. No inversion bridges were found.

T. vulgare var. R.L. 729. All four plants examined had 42 chromosomes. In three plants 21_{II} was the usual configuration, but in the fourth plant most nuclei contained from two to eight univalents. Two plants, including the fourth mentioned above, were studied at the tetrad stage. In the first, 6% of the tetrads had one or two micronuclei. In the irregular plant 70% of the tetrads had from 1 to 10 micronuclei; one inversion bridge was found in eight tetrads, two bridges in two cells, and three in one cell.

B. 50 Hybrid Lines

A summary of the chromosome numbers found in the 336 plants examined in 1937 is given in Table II. Details concerning generations and number of lines are also included.

From the data included in Table II it is seen that less than 42% of the plants examined contained 42 chromosomes—the Vulgare number. In the two crosses for which material from two generations was available it is obvious that the F_7 showed a decided improvement over the F_6 in respect of the increase in the percentage of plants with 42 chromosomes. It should be remarked that the 12 plants with 28 chromosomes were confined to two lines, and that all plants examined in the two lines had 28 chromosomes. In five other lines (two lines in Iumillo × R.L. 729 F_7, and one in each of Iumillo × Hope F_7, Iumillo × R.L. 729 F_6, and Marquillo × Iumillo F_5) no aneuploids were found—all plants examined having 42 chromosomes, but of course this does not eliminate the possibility that these lines contained some plants with

TABLE II

CHROMOSOME NUMBERS FOUND IN THE 50 LINES

Material	Number of lines	Frequency of plants with the chromosome numbers							Total number of plants	Percentage of plants with 42 chromosomes
		28	38	39	40	41	42	43		
Marquillo × Iumillo F_8	13	12	2	14	8	19	47	—	102	46.0
Iumillo × Hope F_6	5	—	1	4	14	8	1	—	28	3.5
Iumillo × Hope F_7	12	—	—	9	26	25	21	1	82	25.6
Marquis × Iumillo F_7	9	—	—	—	1	22	26	1	50	52.0
Iumillo × R.L. 729 F_6	7	—	—	—	1	22	21	2	46	45.6
Iumillo × R.L. 729 F_7	4	—	—	—	—	2	24	2	28	85.7
Totals	50	12	3	27	50	98	140	6	336	41.6

more or less than the Vulgare number of chromosomes. None of the five lines was free from chromosomal aberrations.

In 12 lines no 42-chromosome plants were found. While some of these lines may have contained euploid plants that were not examined, the chromosome numbers found in others make it seem unlikely that any of the plants in them have the Vulgare number (e.g., Line 15 with one 39- and six 40-chromosome plants; Line 16 with four 40-chromosome plants; Lines 22 and 23 with one 39- and four 40-chromosome plants each; and Line 42 with one 38- and six 39-chromosome plants).

The following chromosomal aberrations were found at the first meiotic division:

(a) A chromosome deficient for one complete arm (but never without its "normal" homologue) in 14 plants,

(b) An isochromosome (having a deficiency of one arm and a duplication of the other) in 8 plants,

(c) An association of four chromosomes forming a chain in 16 plants,

(d) An association of four chromosomes forming a ring in 10 plants,

(e) Two associations of four chromosomes (at least one forming a ring) in 2 plants.

(f) An association of three chromosomes forming a chain in 18 plants.

(g) Two associations of three chromosomes forming chains in 2 plants.

(h) An inversion bridge and fragment in 3 plants.

The 73 aberrations were found in 55 of the 336 plants (16.4%). They were distributed among 32 of the 50 lines (Table III).

From the practical standpoint, it may be mentioned that 81 of the 140 plants with 42 chromosomes appeared to be quite stable cytologically. The 81 plants appeared in 26 of the 50 lines and represented all four crosses. From the 81 plants more than 6,000 seeds were forwarded to Dr. Peterson at the Dominion Rust Research Laboratory to be used in establishing new rust-resistant Vulgare lines. Of the 12 plants with 28 chromosomes, six were

TABLE III

DISTRIBUTION OF PLANTS WITH ABERRATIONS FOUND AT THE FIRST MEIOTIC DIVISION

Cross and generation	Total plants studied	Number of plants with chromosome aberrations	Total number of lines	Number of lines in which aberrations were found
Marquillo × Iumillo F_5	102	14	13	7
Iumillo × Hope F_6	28	4	5	3
Iumillo × Hope F_7	82	12	12	6
Marquis × Iumillo F_7	50	15	9	8
Iumillo × R.L. 729 F_6	46	6	7	5
Iumillo × R.L. 729 F_7	28	4	4	3
Totals	336	55	50	32

fertile, and 79 seeds of these plants (which had genes for the Vulgare condition of several characters (22)) were also forwarded to Dr. Peterson for the purpose of establishing partially Vulgare-like lines to be used as parents in further hybridization work.

The distribution of the plants studied at the young pollen tetrad stage is given in Table IV. Included in the table is the distribution of plants that were found to be heterozygous for one or more inverted regions, as evidenced by the presence of inversion bridges and fragments. It is seen from Table IV that 90 of the 175 plants (51.4%) were heterozygous for one or more inversions.

An inversion bridge was seen one or more times in 82 plants, as follows:

No. of tetrads in which a bridge appeared	1	2	3	4	5	6	7	8	9	11
No. of plants	29	13	14	4	7	4	5	3	2	1

Two inversion bridges were seen once in each of seven plants, and one bridge was seen in them as follows:

No. of tetrads in which one bridge appeared	0	5	7	9	14
No. of plants	2	2	1	1	1

Three bridges were seen in one tetrad of one plant, and in it nine tetrads contained one bridge. The 90 plants were distributed among 42 of the 49 lines represented in the tetrad studies (Table IV).

Five-celled "tetrads" were found occasionally in 22 plants.

The percentage of tetrads with one or more micronuclei varied from 0 to 100 in different plants (Table V). There is an increasing frequency, on the average, of irregular tetrads in plants with 0, 1, 2, and 3 univalents. But

TABLE IV

DISTRIBUTION OF PLANTS HETEROZYGOUS FOR ONE OR MORE INVERSIONS

Cross and generation	Total plants studied	Number of plants with inversions	Total number of lines	Number of lines in which inversions were found
Marquillo × Iumillo F_5	56	33	13	13
Iumillo × Hope F_6	15	6	5	4
Iumillo × Hope F_7	48	21	12	11
Marquis × Iumillo F_7	21	5	8	4
Iumillo × R. L. 729 F_6	20	16	7	7
Iumillo × R.L. 729 F_7	15	9	4	3
Totals	175	90	49	42

the great variability, from plant to plant, in the frequency of the "loss" of the univalent in plants of the 41-chromosome group is to be noted. Plants with 20 bivalents were as stable cytologically as those with 21 bivalents. There is a significantly lower percentage of irregular tetrads in plants of the 39-chromosome group with 19 bivalents and 1 univalent than in those with 18 bivalents and 3 univalents; this situation also obtains between plants of the 40-chromosome group with 20 bivalents and those with 19 bivalents and 2 univalents.

Details of the occurrence of aberrant pollen mother-cells (with less than the $2n$ number of chromosomes) in 16 of the 336 hybrid derivatives have already been published (14).

TABLE V

VARIATION IN THE PERCENTAGE OF TETRADS WITH MICRONUCLEI IN PLANTS WITH DIFFERENT CHROMOSOME NUMBERS

Percentage of tetrads with micronuclei	Frequency of plants with the chromosome numbers									Total plants
	28	38	39		40		41	42	43	
			1_I	3_I	0_I	2_I				
0 to 10	4	—	1	—	12	—	1	70	1	89
11 to 20	2	—	2	—	—	—	6	7	—	17
21 to 40	—	—	3	—	—	4	15	2	—	24
41 to 60	—	—	—	1	—	5	15	1**	1	23
61 to 80	—	—	—	3	—	4	6	—	—	13
81 to 100	—	1	—	3*	—	4	—	—	1	9
Total plants	6	1	6	7	12	17	43	80	3	175
Mean % for group	8.5	90.0	22.4	83.7	5.4	63.2	45.7	6.3	45.0	

* *Including one plant in which less than 5_I were not seen.*

** *Plant contained an isochromosome.*

Plants with 28 Chromosomes

First division. 12 plants. No multivalents were found, 14_{II} occurring quite regularly.

Tetrad. 6 plants. The percentage of tetrads with one or two micronuclei varied from 2 to 15, the mean being 8.5%. In each of three plants, an inversion bridge was detected in three pollen mother-cells.

Plants with 38 Chromosomes

First division. 3 plants. One had $17_{II} + 4_{I}$, one had $18_{II} + 2_{I}$, and the third had 1 $chain_{IV} + 16_{II} + 2_{I}$ in 25% of the metaphase nuclei.

Tetrad. 1 plant ($17_{II} + 4_{I}$). From one to six micronuclei were found in 90% of the tetrads.

Plants with 39 Chromosomes

First Division. 27 plants. Plants of this group fall into five categories with respect to the least number of univalents observed in some nuclei:

(a) 3 plants with no univalents—$1_{III} + 18_{II}$. An association of three chromosomes was seen in 5%, 10% and 25% of the nuclei in the three plants, respectively.

(b) 7 plants with one univalent—$19_{II} + 1_{I}$. In one plant (see Parent 1, "1938 Observations") the univalent was an isochromosome.

(c) 1 plant with two univalents—$1_{III} + 17_{II} + 2_{I}$. The association of three chromosomes was observed in 25% of the nuclei.

(d) 15 plants with three univalents. The typical metaphase arrangement was $18_{II} + 3_{I}$. But in two plants, four chromosomes were associated to form a chain in 3% and 6% of the nuclei. A third plant contained an unequal bivalent, i.e., one chromosome consisted of only one arm.

(e) 1 plant with five univalents—$17_{II} + 5_{I}$. Nuclei with seven and nine univalents were frequent. Cells with three univalents or less were not seen.

Tetrad. 13 plants. Five plants were heterozygous for at least one inverted region.

(a) 1 plant with $1_{III} + 18_{II}$. From one to four, but usually one or two, micronuclei occurred in 15% of the pollen mother-cells. An inversion bridge was seen in eight tetrads.

(b) 5 plants with $19_{II} + 1_{I}$. The percentage of tetrads with from one to three micronuclei varied from 10 to 31, the mean being 22.4%. An inversion bridge was seen eight times in one plant. Four 5-celled "tetrads" were seen in one plant.

(c) 5 plants with $18_{II} + 3_{I}$. The percentage of tetrads with from one to six micronuclei varied from 53 to 85, the mean being 73.0%. An inversion bridge was detected four times in one plant, six times in another and seven times in a third.

(d) 1 plant with 1 $chain_{IV} + 16_{II} + 3_{I}$. From one to six micronuclei were seen in 78% of the tetrads.

(e) 1 plant with $17_{II} + 5_{I}$. From 1 to 10 micronuclei were observed in 100% of the tetrads.

Plants with 40 Chromosomes

First division. 50 plants. Plants of this group fall into two classes in respect of the least number of univalents observed in some nuclei:

(a) 19 plants with no univalents. One plant had 1 $ring_{IV} + 18_{II}$ in 6% of the nuclei examined. In all plants 20_{II} was the typical configuration, although as expected two or more univalents were sometimes observed. (See Parents 2, 3, 4, and 5.)

(b) 31 plants with two univalents. In one plant, 4% of the nuclei had 1 $chain_{IV} + 17_{II} + 2_{I}$, but the usual configuration in all was $19_{II} + 2_{I}$. In two plants, one of the univalents was an isochromosome.

Tetrad. 29 plants. Sixteen plants were heterozygous for at least one inversion.

(a) 11 plants with 20_{II}. The percentage of tetrads with one or two micronuclei varied from 0 to 9, the mean being 5.9%. An inversion bridge was seen once in two plants, twice in two, and three times in a fifth plant. In a sixth plant two bridges were seen in one tetrad. One or two 5-celled "tetrads" were observed in three plants.

(b) 1 plant with 1 $ring_{IV} + 18_{II}$. No micronuclei were seen in 100 tetrads.

(c) 16 plants with $19_{II} + 2_{I}$. The percentage of tetrads with from one to six micronuclei varied from 21 to 99, the mean being 62.2%. An inversion bridge was seen one, three, four, five, and seven times in three, two, one, one, and two plants, respectively. One or two 5-celled "tetrads" were observed in three plants.

(d) 1 plant with 1 $chain_{IV} + 17_{II} + 2_{I}$. From one to six micronuclei were found in 80% of the tetrads. An inversion bridge was detected nine times.

Plants with 41 Chromosomes

First division. 98 plants. Plants of this group fall into three classes with respect to the least number of univalents observed in some nuclei:

(a) 6 plants with no univalents. In one plant an association of three chromosomes was seen in 7 of the 21 nuclei examined; an association of three and one of four chromosomes (both chains) occurred in one nucleus. In five plants an association of three chromosomes was seen in 6%, 20%, 20%, 22%, and 44% of the nuclei, respectively. In all six plants, from one to nine unpaired chromosomes were observed in some nuclei.

(b) 90 plants with one univalent (one of them Parent 7). In these plants the usual arrangement was $20_{II} + 1_{I}$. Four chromosomes formed a ring or figure-of-eight in 10% of the nuclei of one plant. A chain of four chromosomes appeared in 3%, 3% and 5% of the nuclei in three plants, respectively. In another plant 5% of the nuclei contained a chain of four chromosomes and 7% a chain of three chromosomes. In five plants there was an unequal

bivalent (see Parent 6). In three plants the univalent was an isochromosome.

(c) 2 plants with two univalents. In 3% and 4%, respectively, of the nuclei in the two plants an association of three chromosomes was seen. Nuclei with less than two unpaired chromosomes were not found.

Tetrad. 43 plants. Twenty-four plants were heterozygous for one or more inversions.

(a) 1 plant with $1_{III} + 19_{II}$. From one to three micronuclei were found in 34% of the tetrads. An inversion bridge was detected in five pollen mother-cells.

(b) 1 plant with 1 $chain_{IV} + 1_{III} + 17_{II}$. From one to five micronuclei were seen in 21% of the tetrads. An inversion bridge was seen three times.

(c) 2 plants with 1 $chain_{IV} + 18_{II} + 1_{I}$. From one to six micronuclei were observed in 31% and 54% of the tetrads, respectively. In the first plant an inversion bridge was seen five times. In the second plant one bridge was seen in five tetrads and two bridges were seen in two tetrads.

(d) 39 plants with $20_{II} + 1_{I}$. The percentage of tetrads with from one to four micronuclei ranged from 5 to 74, the mean being 42.1%. An inversion bridge was detected once in seven plants, twice in two plants, three times in two plants, six times in one plant, seven times in two plants, eight times in one plant, and nine times in one plant. Two inversion bridges were seen once in each of three plants (5, 7, and 9 tetrads, respectively, contained one bridge). In the nineteenth plant to show bridges, three were found in one tetrad (9 tetrads contained one bridge). One or two 5-celled "tetrads" were observed in five plants.

Plants with 42 Chromosomes

First division. 140 plants. Seven different types of plants were found:

(a) 120 plants with 21_{II}. In these plants no multiple associations of chromosomes were detected. The maximum number of univalents in different plants varied from 2 (Parent 18) to 12. Four plants (one of them Parent 15) were characterized by an unequal bivalent; in one of them the two heteromorphic chromosomes formed a bivalent in only half the nuclei. An isochromosome was present in two plants (one of them Parent 16); in each case both arms of the aberrant chromosome were homologous with one arm of its "normal" homologue.

(b) 8 plants with 1 $ring_{IV} + 19_{II}$. In four plants, respectively, the association of four appeared as a ring in 3%, 7%, 10% (Parent 11), and 20% of the nuclei. In one plant a ring of four chromosomes was found in 4%, a chain of four in 8%, and a a chain of three in 36%, of the nuclei. In the sixth plant a ring of four (14% of the nuclei), a chain of four (18%), and a chain of three chromosomes (18%) were observed (Parent 10). In the seventh plant a ring of four and a chain of four chromosomes occurred in 30 and 10% of the nuclei, respectively: this plant also contained an unequal bivalent. Finally, in one individual a ring of four and a chain of three chromosomes were observed in 21% and 45% of the nuclei, respectively (Parent 14).

(c) 6 plants with 1 $chain_{IV} + 19_{II}$. In five plants, respectively, 10%, 10%, 10%, 13% (Parent 12), and 20% of the nuclei contained a chain of four chromosomes. In the sixth plant a chain of four and a chain of three chromosomes were seen in 19 and 3% of the nuclei, respectively (Parent 13).

(d) 2 plants with $2_{IV} + 17_{II}$. In the first plant a ring of four and a chain of four chromosomes (3% of the nuclei), a ring of four (30%), and a chain of four (28%) were observed. In the second plant (Parent 8), a ring of four and a chain of four chromosomes (9% of the nuclei), a ring of four (9%), a chain of four (27%), and a chain of three (9%) were observed.

(e) 1 plant with 1 $ring_{IV} + 1_{III} + 17_{II} + 1_{I}$. A ring of four and a chain of three chromosomes (7% of the nuclei), a ring of four (14%) and a chain of three (14%) were seen. In this plant there was an unequal bivalent.

(f) 2 plants with $1_{III} + 19_{II} + 1_{I}$. The association of three chromosomes was seen in 8% and 60% of the nuclei in the two plants. Associations of four chromosomes were not detected.

(g) 1 plant with $2_{III} + 18_{II}$ (Parent 9). Two associations of three were seen once, and one association of three chromosomes occurred 14 times in the 50 nuclei examined. Associations of four or more chromosomes were not observed.

Tetrad. 80 plants. Thirty-eight plants were heterozygous for one inversion and two plants were heterozygous for two inversions.

(a) 71 plants with 21_{II}. The percentage of tetrads with from one to seven micronuclei ranged from 0 to 29, the mean being 5.9%. An inversion bridge was detected once in 15 plants, twice in 6 plants, three times in 3 plants, four times in 2 plants, five times in 4 plants, and six times in 2 plants. Two bridges were seen once in 2 plants, in one of which one bridge was observed in 14 tetrads. One or two 5-celled "tetrads" were seen in 9 plants.

(b) 1 plant with an isochromosome. From one to four micronuclei occurred in 45% of the tetrads.

(c) 5 plants with 1 $ring_{IV} + 19_{II}$. The percentage of tetrads with one or two micronuclei varied from 1 to 4, the mean being 2.5%. An inversion bridge was seen once in one plant, twice in one plant, and three times in two plants.

(d) 3 plants with 1 $chain_{IV} + 19_{II}$. The percentage of tetrads with from one to seven micronuclei in the three plants was 6, 8, and 10 respectively. An inversion bridge was seen once in one plant and twice in a second plant.

Plants with 43 Chromosomes

First division. 6 plants. In one plant no multiple associations were found; from one to seven unpaired chromosomes appeared in different nuclei. The remaining plants were of three types.

(a) 3 plants with $1_{III} + 20_{II}$. A chain of three chromosomes was seen in 8%, 20%, and 33% of the nuclei in the three plants, respectively. An unequal bivalent was present in one plant.

(b) 1 plant with 1 $chain_{IV}$ + 19_{II} + 1 univalent isochromosome (Parent 17). A chain of four chromosomes was observed in 39% of the nuclei, and a chain of three (not involving the isochromosome) in 4% of the nuclei.

(c) 1 plant with 2_{III} + 18_{II} + 1_{I}. One and two chains of three chromosomes were seen in 50 and 10% of the nuclei, respectively. Associations of four or more chromosomes were not observed in the 70 nuclei examined.

Tetrad. 3 plants. Two plants were heterozygous for an inversion.

(a) 2 plants with 1_{III} + 20_{II}. Three per cent and 47% of the tetrads, respectively, contained from one to three micronuclei. In the latter plant an inversion bridge was seen in two tetrads, and one 5-celled "tetrad" was found.

(b) 1 plant with 2_{III} + 18_{II} + 1_{I}. From one to five micronuclei were observed in 85% of the tetrads. An inversion bridge was detected in eleven tetrads.

1938 Observations

(For complete details of *Parents*, see under "1937 Observations".)

Parent 1. $2n = 39$ (19_{II} + 1 univalent isochromosome). Micronuclei were present in 17% of the tetrads.

Progeny: Two 40-chromosome plants. One had 20_{II}, including an unequal bivalent, and 11% of the tetrads had micronuclei. The second plant had 1 $chain_{IV}$ + 18_{II} in 10% of the metaphase nuclei and 10% of the tetrads were irregular. No isochromosome was detected in the two plants.

Parents 2, 3, 4, 5. $2n = 40$ (20_{II}). In these plants from 4 to 7% of the tetrads had micronuclei.

Progeny: Three 40-chromosome plants (20_{II}) with micronuclei in from 4 to 5% of the tetrads. One 41-chromosome plant (20_{II} + 1_{I}) heterozygous for at least one inversion.

Parent 6. $2n = 41$ (20_{II} + 1_{I}, including an unequal bivalent).

Progeny: Five 41-chromosome plants (20_{II} + 1_{I}). One plant with a univalent isochromosome had micronuclei in 68% of the tetrads. One plant was heterozygous for an inversion.

Parent 7. $2n = 41$ (20_{II} + 1_{I}). This plant was heterozygous for an inverted region.

Progeny: Three plants, two of which had 41 chromosomes (20_{II} + 1_{I})—first division material was not available for the third plant. Two plants were heterozygous for an inversion. The percentage of tetrads with micronuclei in the three plants was 60, 42, and 19, respectively.

Parent 8. $2n = 42$ (2_{IV} + 17_{II}). At least one association of four chromosomes was a ring.

Progeny: One 42-chromosome plant. Three and four chromosomes were associated to form chains in 6 and 12%, respectively, of the nuclei at first metaphase; eight univalents were seen once; and 54% of the tetrads were irregular. One 43-chromosome plant (1_{III} + 20_{II}). The chain of three

chromosomes appeared in 20% of the first metaphase nuclei; and 63% of the tetrads were irregular.

Parent 9. $2n = 42\ (2_{III} + 18_{II})$.

Progeny: One (probably two—first division material was lacking for the second plant) 42-chromosome plant, with three and four chromosomes associated to form chains in 10 and 30%, respectively, of the nuclei; 8% of the tetrads had micronuclei; in the second plant 4% of the tetrads were irregular. One 43-chromosome plant with a chain of three and a chain of four chromosomes (20% of the nuclei), a chain of four (20%) and a chain of three (10%); 68% of the tetrads were irregular.

Parent 10. $2n = 42\ (1\ ring_{IV} + 18_{II})$. A ring of four (14% of the nuclei), a chain of four (18%), and a chain of three (18%) were seen. A micronucleus was found in 2% of the tetrads.

Progeny: Three 42-chromosome plants, one with a chain of four in 30% of the nuclei, and two with 21 bivalents. In the two latter plants 3% and 4% of the tetrads had micronuclei.

Parent 11. $2n = 42\ (1\ ring_{IV} + 19_{II})$. One or two micronuclei appeared in 3% of the tetrads, and an inversion bridge was seen once.

Progeny: Four 42-chromosome plants (21_{II}), two of which were heterozygous for an inversion; the percentage of irregular tetrads in three of the plants was 2, 5, and 15, respectively. One 43-chromosome plant, with a chain of three and 20 bivalents in 63% of the nuclei; an inversion bridge was seen four times, and 35% of the tetrads contained micronuclei. In the sixth plant ($2n = ?$) there was an inversion, and 19% of the tetrads were irregular.

Parent 12. $2n = 42\ (1\ chain_{IV} + 19_{II})$. A micronucleus appeared in 6% of the tetrads.

Progeny: Three 42-chromosome plants. The first had a chain of four chromosomes in 60% of the nuclei, and 4% of the tetrads were irregular. Two plants had 21 bivalents, and 2% and 6% of the tetrads had micronuclei; an inversion bridge was seen in the latter plant. The fourth plant probably had 42 chromosomes since only 6% of the tetrads were irregular.

Parent 13. $2n = 42\ (1\ chain_{IV} + 19_{II})$.

Progeny: One 41-chromosome plant with a chain of three and 19 bivalents in 19 of 20 nuclei examined; 3% of the tetrads had one micronucleus. One 42-chromosome plant in which a ring of four (15% of the nuclei), a chain of four (45%) and a chain of three chromosomes (10%) were seen; 3% of the tetrads had a micronucleus.

Parent 14. $2n = 42\ (1\ chain_{IV} + 19_{II})$.

Progeny: One 42-chromosome plant with a ring of four and a chain of three chromosomes appearing in 20 and 60%, respectively, of the nuclei; 11% of the tetrads had one or two micronuclei and an inversion bridge was seen once.

Parent 15. $2n = 42\ (21_{II}$, including an unequal bivalent).

Progeny: Two 42-chromosome plants (21_{II}). One had an unequal bivalent. The other had a micronucleus in 4% of the tetrads.

Parent 16. $2n = 42$ (21_{II}). This plant had an isochromosome which occasionally paired with one arm of its normal homologue.

Progeny: One 41-chromosome plant with 20 bivalents and a univalent isochromosome. Two 42-chromosome plants, one with 21 bivalents and the other with a chain of four chromosomes in 85% of the nuclei; the percentage of irregular tetrads in the two plants was 5 and 2, respectively.

Parent 17. $2n = 43$ (1 $chain_{IV}$ + 19_{II} + 1 univalent isochromosome). A chain of four and a chain of three chromosomes (not involving the isochromosome) appeared in 39 and 4%, respectively, of the nuclei.

Progeny: Three 42-chromosome plants (21_{II}). In one, five inversion bridges and three fragments were observed in a second anaphase pollen mother-cell, which was too early to be included in the tetrad count; and 5% of the tetrads had from one to three micronuclei. The second plant had an isochromosome, and 16% of the tetrads were irregular. In the third plant, 7% of the tetrads had one or two micronuclei.

Parent 18 (Check). $2n = 42$ (21_{II}). One or two micronuclei were found in 3% of the tetrads.

Progeny: Four 42-chromosome plants (21_{II}). An average of 3.10% of the tetrads in the four plants contained one or two micronuclei.

General Considerations

Aneuploid Plants

Approximately one-half (54.8%) of the plants examined in 1937 were aneuploids, and they were found in 43 of the 50 lines. This is an indication of the problem that confronts the plant breeder in obtaining euploid plants of the desired type by phenotypic selection from heteroploid hybrids in wheats at least. The author knows of no other cytological study on comparable material, so that it is not known whether this ratio of aneuploids to euploids in *selected* descendants of pentaploid hybrids is typical.

Kihara (10) reported that the chromosome formula of most plants with more than 14 bivalents is $(14 + x)$ bivalents + $(7 - x)$ univalents. Thus, plants with 38, 39, 40, and 41 chromosomes should show $17_{II} + 4_{I}$, $18_{II} + 3_{I}$, $19_{II} + 2_{I}$, and $20_{II} + 1_{I}$, respectively, in the first metaphase of the reduction divisions. A few that did not conform to this classification, for instance, plants with $20_{II} + 0_{I}$, he called "sterile combinations" because they set no seeds or the seeds germinated poorly or not at all.

Thompson and Cameron (27) found that observation of the number of univalents (in Emmer × Vulgare F_1 backcrosses to the Vulgare wheats Chinese and Marquis) was all that was necessary, since the total number of bivalents observed in each plant proved to be the same as that determined by subtracting the number of univalents from 21, e.g., if four univalents were observed the number of bivalents observed was 17 (= 21 − 4).

On the other hand, Thompson and Hollingshead (28), in a study of derivatives of *T. vulgare* × *T. dicoccum*, reported the occurrence of a 37-chromosome plant with $17_{II} + 3_{I}$, rather than the "expected" $16_{II} + 5_{I}$.

Sears (26) studied offspring of a cross of a "haploid" *T. vulgare* var. Chinese Spring × a "diploid" *T. vulgare*. He found two 41-chromosome plants with two univalents ($1_{III} + 18_{II} + 2_{I}$), and among the progeny of a 42-chromosome plant ($1_{III} + 19_{II} + 1_{I}$) he obtained a plant with 20_{II}, which "although small and lacking in vigor had normal pollen fertility and set a number of selfed seeds."

In the present study, a considerable number, 41, of the 178 plants with 38, 39, 40, and 41 chromosomes did not have the number of univalents expected under Kihara's scheme. Most of these set seeds, and several of the lines with 20_{II} have been grown on (Parents 2, 3, and 4). Of the three plants with 38 chromosomes, two had two univalents in some nuclei. Of the 27 plants with 39 chromosomes, three, seven, one, and one had 0, 1, 2, and 5 univalents, respectively. Of the 50 plants with 40 chromosomes, 19 had 0 univalents. And of the 98 plants with 41 chromosomes, six and two had 0 and 2 univalents, respectively. Thus, it is clear that no simple relation obtains between numbers of bivalents and univalents in wheats derived from pentaploid hybrids involving Iumillo. Photomicrographs of first metaphase nuclei with $19_{II} + 1_{I}$ and with 20_{II}, as well as the "expected" types with $18_{II} + 3_{I}$ and $19_{II} + 2_{I}$ have been previously published by the author (14).

The data obtained from the tetrad studies corroborate the chromosome pairing relationships found in plants with 39 and 40 chromosomes. As expected, the seven plants with $18_{II} + 3_{I}$ were much more irregular than the six with $19_{II} + 1_{I}$ (Table V). Similarly, the 17 plants with $19_{II} + 2_{I}$ were highly irregular as compared with the 12 which had 20_{II}.

The 41-chromosome plants varied greatly in the percentage of tetrads with micronuclei. In other words univalents are included in the daughter nuclei of tetrads with varying frequencies in different individuals. There can be little doubt but that the divergent speltoid and fatuoid ratios (5, 6, 7, and 19) obtained in certain 41-chromosome lines are due, in part at least, to this phenomenon.

Segmental Deficiencies

Chromosomes deficient for one complete arm were found in 14 of the 336 plants examined in 1937. Prophase configurations of aberrant chromosomes have not been seen, but the metaphase (Fig. 1) and anaphase (Fig. 2) appearance indicates strongly that the attachment is terminal. The short chromosome was transmitted from Parent 15 (21_{II}, including an unequal bivalent) to at least one of its offspring that had the same chromosome constitution as the parent.

Darlington (1) considers that "In plants generally we may say that there is no undoubted evidence of chromosomes with terminal centromeres forming

part of the permanent complement of a species". Such chromosomes have been produced by McClintock (19) and Rhoades (24) by X-raying maize plants. Love (16) has found certain speltoids of hybrid origin to be heterozygous for the loss of one complete arm of a chromosome. Further, Love (13) has shown that white chaff off-types in a golden chaff winter wheat are homozygous for such a deficiency; these plants have now bred true for five generations (unpublished data).

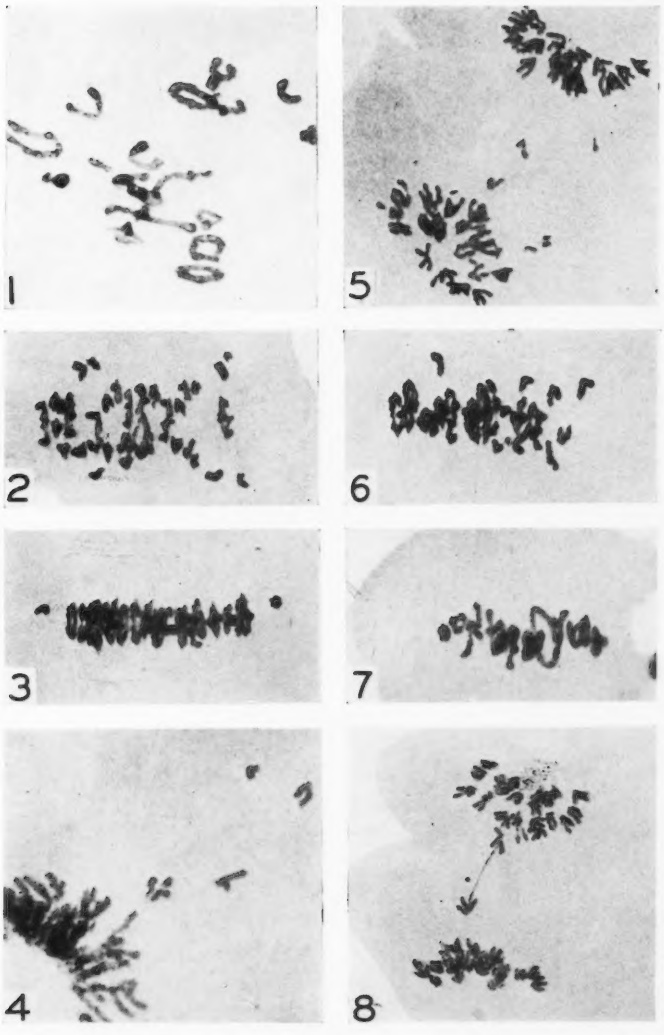
One chromosome with a terminal attachment region arose in the course of this study. Parent 1, with a univalent isochromosome, produced at least one plant (20_{II}, including an unequal bivalent) containing a chromosome with a terminal attachment region. This probably arose as a result of a break at the attachment region of the isochromosome in an embryosac mother-cell. The offspring probably resulted from a natural cross, and got the normal homologue from a nearby plant.

Segmental Duplications

Isochromosomes were found in eight plants in 1937. In four plants, these "secondary" chromosomes frequently paired with one arm of the normal homologue. In such cases disjunction at first anaphase was usually quite regular. Failure of pairing with the normal mate (Fig. 3) often resulted in breaks at the attachment region (Figs. 4 and 5) producing chromatid arms with terminal attachments, or three chromatids with a common attachment and one chromatid with no attachment. In one 43-chromosome plant, although the "extra" chromosome was an isochromosome, a "secondary trisomic" configuration was not seen.

In two instances (see Parents 16 and 17) an isochromosome was transmitted from parent to offspring. It seems that in wheat an isochromosome, and therefore deficiency-duplication gametes, can survive (see also Sears (26)). Parent 1, with an isochromosome, gave rise to a plant with a chromosome deficient for one complete arm (see above, under "Segmental Deficiencies"). A similar case has been previously reported (13).

An isochromosome appeared in one of the progeny ($2n = 41$) of Parent 6 ($2n = 41$, with an unequal bivalent). The isochromosome probably resulted from a duplication of the one arm of the deficient chromosome. The occurrence of isochromosomes in monosomic wheat has been reported by Håkansson (3), but they were then thought to be the result of crossing over in an intercalary duplication near the attachment of the "C" chromosome. Huskins and Smith (unpublished data) have obtained a chromosome consisting of the major arm of the "C" chromosome with a terminal attachment, in progeny of a plant with a "secondary" chromosome consisting of two major arms of the "C" chromosome. Rhoades (23) found that maize plants hyperploid for the short arm of chromosome V with a terminal attachment region produced a few aberrant offspring with an isochromosome. He suggested that "occasionally the 'secondary' chromosome is produced by a doubling of the half-chromosome fragment. Reduction takes place in the succeeding anaphase.



Photomicrographs of pollen mother-cells in vulgare-like derivatives of pentaploid wheat hybrids. Aceto-carmine preparations. Figs. 1 and 4 ×1100, others ×560.

FIG. 1. *Part of a first metaphase nucleus with a univalent, an association of three chromosomes, and an unequal bivalent. Note the terminal position of the attachment region in the short chromosome of the latter.* FIG. 2. *Mid-anaphase in another plant. Members of the unequal bivalent have disjoined (at right of cell).* FIGS. 3, 4, AND 5. *Behaviour of isochromosomes.* FIG. 3. *First metaphase. Isochromosome at right, normal homologue at left of cell.* FIG. 4. *Part of a first anaphase pollen mother-cell in the same plant. Three chromatid arms of the isochromosome with a common attachment, the fourth with no attachment.* FIG. 5. *Another first anaphase pollen mother-cell in the same plant. Three chromatid arms of the isochromosome with a common attachment, the fourth with a terminal attachment.* FIG. 6. *First metaphase. Note the alternate disjunction of the members of the association of four chromosomes.* FIG. 7. *First metaphase. A chain of four and a ring of four chromosomes.* FIG 8. *First anaphase. An inversion bridge and an accompanying fragment.*

One pole receives no fragment chromosome while the other receives both fragments which have become incorporated into a single chromosome with a median attachment region."

Associations of Three and Four Chromosomes

One and two associations of three chromosomes were found in 18 and 2 plants, respectively. In six plants ($2n = 41$, with $1_{III} + 19_{II}$) the association of three was apparently due to a reciprocal translocation (the fourth member being absent in the aneuploid). The one which occurred in 44% of the nuclei may have been a trisome. One, two, three, and one plants, respectively, with 39, 41, 42, and 43 chromosomes contained one or two associations of three chromosomes, but a ring or chain of four was not found. Sears (26) found several instances of the same phenomenon.

One (Fig. 6) and two (Fig. 7) associations of four chromosomes appeared in 26 and 2 plants, respectively. The four chromosomes formed a ring in 10 and a chain in 16 plants. In some plants the association of four was frequently replaced by an association of three and a univalent, two bivalents, a bivalent and two univalents, or four univalents. It is assumed that the associations of four chromosomes are due to reciprocal translocations, the chain configurations resulting from a smaller interchange than the rings.

The reciprocal translocations may have resulted from crossing over in the F_1 hybrids between "non-homologous" chromosomes having small regions in common (compare 6, 11, 12, and 26). Multivalent configurations are frequent in F_1 pentaploid wheat hybrids (Love, unpublished data).

A chain of four chromosomes was found in parent and progeny in one case (see Parent 12), and probably in another (Parent 13) in which a chain of three was found in a 41-chromosome plant. A chain of four appeared in Parents 13 and 14 and a ring of four in one of the progeny of each. A ring (Parent 11) and a chain (Parent 17) of four chromosomes, though present in the parents, were not found in the few progeny examined. A ring of four was detected in Parents 8 and 10, but chains of four appeared in the progeny. Multiple associations were not seen in Parents 1 and 16, but chains of four appeared in one of the progeny of each; they were probably the result of natural crossing. Parent 9, with two chains of three chromosomes, gave rise to two plants, each with a chain of four chromosomes; the 42-chromosome offspring with a chain of four probably resulted from a natural cross.

The first anaphase observations of the regular disjunction of the associations of four chromosomes are corroborated by the fact that plants containing such associations have, in general, a percentage of tetrads with micronuclei no higher than have their "normal" sibs. The regular alternate disjunction of members of rings of four chromosomes in wheat has previously been reported by other workers (26, 29). In the present study only one early anaphase configuration was seen in which disjunction was definitely not alternate.

Relative Inversions

Three bridges occurred in a single tetrad in one plant, two in seven plants, and one in 82 plants. Inversion heterozygotes have been previously reported in wheat on only two occasions: in *Triticum monococcum* by Yasui (30) and in F_1 *T. dicoccum* × *T. monococcum* by Mather (17).

In three plants one inversion bridge and an accompanying fragment (Fig. 8) were seen at first anaphase. Second division material was not available for these plants so it is impossible to compare the frequencies of bridges at the two stages.

One second division bridge is the result of one (or two) cross-overs within the inverted region together with a second (or third) cross-over between the inversion and the attachment region (20). Since two second division bridges may appear in one pollen mother-cell as the result of two comparate chiasmata within the same inverted segment, the presence of two bridges in a tetrad cannot be taken as evidence for two different inversions; especially is this so since the several micronuclei usually present in a tetrad make it difficult to identify inversion fragments. The 56 plants in which one bridge was seen three times or fewer, as well as the two plants in which two bridges were detected once, may have been heterozygous for only one inverted region. But the following 32 plants were probably heterozygous for two or more inversions: the 26 plants in which one bridge was seen from four to eleven times (in 100 tetrads), the five plants in which two bridges were detected once and one bridge was seen from five to fourteen times, and the one plant in which three bridges were detected once and one bridge was seen nine times.

Inversion bridges were found in parents and progeny in two cases (see Parents 7 and 11). Unfortunately, the limitations to an exact cytological analysis of inversions in wheat are such that it is impossible to be certain that the inversions detected among the progeny of the two plants were the same ones found in the parents. No inversion bridges were detected in the 100 young pollen tetrads examined in Parent 5 ($2n = 40$) although they were in one of the progeny ($2n = 41$) which was probably the result of a natural cross. Second division material was not available for Parents 6, 8, 14, and 17; inversion bridges were detected in some of the progeny of each, and the parents may have been heterozygous for one or more inversions.

Natural Crossing

The cytological evidence, reviewed above, indicates that of the 43 progeny of the seventeen irregular plants, at least six (13.9%) resulted from natural crossing: two progeny of Parent 1, and one of the progeny of each of Parents 5, 9, 12, and 16. Goulden and Neatby (2) found that some lines of Marquillo gave as high as 50% of natural crossing, and that the frequency was associated with self-sterility. Hayes and Garber (4) reported that the incidence of natural crossing in standard varieties of wheat varied from two to three per cent. Although the number of individuals tested in the present study is small,

the results suggest that the cytological irregularities are responsible for the natural crossing that occurred.

Discussion

Disregarding irregularities in chromosome number, the 336 hybrid derivatives examined in 1937 may be divided into three categories, on the basis of chromosome behaviour:

(1) Plants comparatively stable cytologically;

(2) Plants with general irregularities, as evidenced by the failure of mating or separation of the members of any of the chromosome pairs, and even by irregularities in somatic divisions (14);

(3) Plants with irregularities confined to one or a few specific chromosomes.

While wide crosses are made in order to produce radical new combinations of genes, stable plants (Type 1 above) are necessary to establish relatively pure-breeding lines. But plants of Type 3 may be very important to the plant breeder, as well as to the worker particularly interested in general cytogenetics. It is obvious that the abnormalities (inversions, translocations, duplications, and deficiencies) are the result of combining diverse, established, viable gene combinations—and they may give rise to further, new viable combinations that are not found in the parent species or stable lines. Such plants, with specific irregularities, should not be overlooked in any breeding program since they may be the means of breaking the linkage between "desirable" and "undesirable" genes, a linkage that in the past has often proved a decided handicap in the attempt to transfer one or a few characters from one variety to another (e.g., Hope and H-44 are rust resistant wheats, but they are susceptible to "head blight" diseases (22)).

From the plant breeding standpoint, the diversity of chromosomal types derived from interspecific or intergeneric hybridization cannot be too strongly emphasized. Only rarely is the plant breeder completely successful in obtaining the desired type by selection from any one particular cross. He finds it necessary to make one or more backcrosses of the derived type on to one or the other parent, or even to cross it with a different variety. The necessity of correlating intensive cytological study with genetic analysis of such hybrids and hybrid derivatives is obvious.

Finally, in varieties such as Hope, Marquillo and R.L. 729 which are known to contain aberrant individuals, the plant breeder would be advised to use as parents plants known to be stable cytologically.

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FLUCTUATIONS IN NUMBERS OF BACTERIA IN SOIL[1]

By Norman James[2] and Marjorie L. Sutherland[3]

Abstract

Comprehensive studies on the relation of moisture, temperature, and time to the numbers of bacteria in a fallow plot were carried on over a period of 15 weeks during the summer of 1939. The data involved represent: moisture—percentages of dry weights of samples; temperature—averages of air temperature readings for three days preceding samplings; time—numbers of days from the beginning of the experiment to dates of sampling; numbers of bacteria—plate count determinations from the following:

On each of 6 dates 5 composite samples, with 48 plates from each.
On each of 6 dates 9 composite samples, with 27 plates from each.
On each of 10 dates 12 composite samples, with 27 plates from each.

On the basis of linear regression, bacteria in millions and moisture in percentage give a significant correlation for between day samples. The same is true for samples plated on the same day.

The application of multiple regression of average numbers of bacteria in the samples handled at each date on average moisture in percentage, temperature in degrees F. and time in days shows that these environmental factors account for most of the variation in numbers of bacteria in the plot during the period of this study.

The correlation between numbers of bacteria and moisture in the samples on different dates was found to be logarithmic. This relation becomes more nearly linear when the correlation is carried out on the numbers of bacteria adjusted for the factor associated with time.

Introduction

The numbers of bacteria obtained from soil by the plating method are known to fluctuate with moisture, temperature, time of sampling, and other factors, and excellent reviews have appeared in the literature. The object of this report is not primarily to add further proof of such relations, but rather to show how these gross environmental factors may mask treatment effects under consideration or introduce spurious ones; and how data may be treated to remove this masking effect in order that a better measure of the effect under consideration may be available.

Experimental

During the summer of 1939 a fallow one-hundredth acre plot on the University farm was sampled at short intervals over a period of 15 weeks in connection with three experiments on sampling and plating technique. The procedures followed were based on work reported earlier (3–5). The number of composite samples taken at random from the plot at one time ranged from 5, with 48 plates from each in the first experiment, to 9, with 27 plates from each

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in the second, and 12 with 27 plates from each in the third. The number of replications of samples, of subsamples, of dilutions from each subsample and of plates from each dilution provides the basis for a more precise estimate of the bacterial population in the plot at one time than is usual in studies of this type. At the end of the season, 22 such estimates were available, each based on the average counts from 240 plates in Experiment 1, 243 plates in Experiment 2, and 324 plates in Experiment 3, respectively. Moisture determinations were made on each sample at the time of plating. These determinations, likewise because of the number of replications at one sampling, provide fair estimates of the moisture content in the soil of the plot sampled. Unfortunately, soil temperature readings were not available for the area over the whole period concerned. Data for temperature relation studies were obtained by averaging the mean air temperature readings for the three days immediately preceding each sampling. Average air and soil temperature readings were correlated for the period extending from June 1 to August 31, and gave a correlation coefficient of .7316.

(a) Correlation Between Moisture and Numbers of Bacteria

It appeared advisable first, to examine the relation of moisture in the soil at the time of sampling to the estimate of the bacterial population from two standpoints. 1. What part of the differences among numbers of bacteria on different days can be accounted for by changes in moisture content? 2. What part of the differences among numbers from different samples from one plot on one day can be accounted for by lack of uniformity in moisture content? Analysis of covariance on the data for bacteria in millions per gram of oven-dried soil and moisture in percentage provides the following information:

Source of variance	D.f.	Variance for moisture	Variance for bacteria	Covariance	Correlation coefficient
Experiment 1. June 13 to 30. (5 samples, each of 6 days)					
*Between day samples	5	23.3809	25.3294	14.8100	0.6086
Within day samples	24	.2071	1.6475	.0413	.0707
Experiment 2. July 7 to Aug. 4. (9 samples, each of 6 days)					
Between day samples	5	344.3493	307.3084	316.6772	.9735
Within day samples	48	1.6258	2.6707	1.2328	.5906
Experiment 3. Aug. 18 to Sept. 30. (12 samples, each of 10 days)					
Between day samples	9	89.9633	113.0198	62.0062	.6155
Within day samples	110	.7789	3.9623	.5847	.3328

The Department of Soils of The University of Manitoba found the soil in the plot to have a moisture equivalent of 36.89. The data on air temperature were supplied by the same department.

** Between day samples represent samples plated on different days. Within day samples represent those plated on the same day.*

Since the total correlation is made up of two effects, it is not included in the above analysis. The comparatively few degrees of freedom available for differences from day to day account for much greater proportions of the variance and covariance than do the larger number attributable to samples on each day.

In this study there was no reason to assume that the response of the bacterial population to changes in moisture differed in the three experiments. Accordingly, the data were combined in one analysis and the results for each experiment given appropriate weights. That is, from the three correlation coefficients for variations in moisture and numbers of bacteria from day to day, a weighted average value of z (1) was obtained, which corresponds to a correlation coefficient of 0.7806. This value approximates the accuracy of a similar correlation coefficient obtained from 16 pairs of estimates. For 14 degrees of freedom the 1% level of significance for the correlation coefficient is .6226 (6). This finding is a confirmation of previous reports that there is a significant correlation between moisture in percentage and bacteria in millions per gram of soil as estimated by the plate method.

Similarly, the correlation coefficients for moisture and bacteria on within day samples were combined; this resulted in a coefficient of .3788, with 177 degrees of freedom. In this case the 1% level of significance is .193. This shows a significant relation between moisture and numbers of bacteria in samples of soil obtained at one time from one plot—a factor that should be considered when the influence of various soil treatments or conditions on numbers of bacteria is being studied.

The difference between the correlation coefficient of .7806 for moisture and bacteria in between day samples and that of .3788 in within day samples suggests that the effect of moisture differs in the two cases. This scarcely seems reasonable. Accordingly, the data yielding the correlation coefficient of .3788 were grouped on the basis of their standard deviations. Then, new correlation coefficients were calculated for each group. The data are presented along with similar data for between day samples, as follows:

Maximum moisture range	Standard deviation	D.f.	Correlation coefficient	Level of significance
Within day samples				
1.64	0 – 0.5	24	0.0434	—
2.96	0.5 – 1.0	62	.2536	5% Pt. 0.2464
6.04	1.0 – 2.0	55	.5138	1% Pt. .3395
Between day samples				
19.34	4.7237	14	.7806	1% Pt. .6226

It is apparent that the size of the correlation coefficient is influenced by the range in moisture among the samples considered: the wider the range in moisture, the larger the coefficient. Obviously, if the samples considered were uniform in moisture the coefficient would be zero, even though there is an actual correlation between moisture and numbers of bacteria when there is variation in the moisture content among the samples used. In this case the range of moisture in the within day samples was less than 3% at 16 samplings, and not above 6.04% at the other 6, while the average moisture for between day samples varied over a range of 19.34%. This explains the apparent discrepancy between the two coefficients.

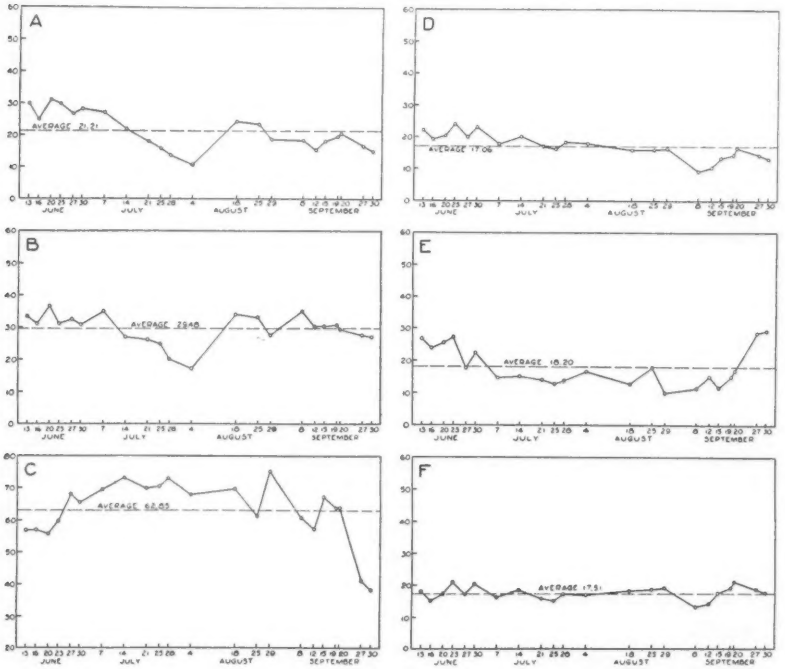

FIG. 1.

X—dates of sampling.

A... *Y—bacteria in millions per gram of oven-dried soil.*

B... *Y—moisture in percentage.*

C... *Y—average air temperature in degrees F.*

D... *Y—differences between numbers of bacteria and theoretical estimates, calculated from regression of numbers of bacteria on moisture, plotted as deviations from an estimate based on a moisture reading of 25%.*

E... *Y—differences between numbers of bacteria and theoretical estimates, calculated from regression of numbers of bacteria on moisture and temperature, plotted as deviations from an estimate based on moisture at 25% and temperature at 65° F.*

F... *Y—differences between numbers of bacteria and theoretical estimates, calculated from regression of numbers of bacteria on moisture, temperature and time, plotted as deviations from an estimate based on moisture at 25%, temperature at 65° F. and time at 55 days.*

(b) Correlation of Moisture, Temperature and Time with Numbers of Bacteria

The results of these analyses appeared consistent enough to warrant a more detailed consideration of moisture and the possible influence of additional factors on the number of bacteria in soil. Accordingly, a new set of data was prepared. Numbers of bacteria in the samples plated at one time were averaged to provide the best available estimate of the population at that time. Likewise, moisture results were averaged. These data were used without weighting for such factors as number of samples, subsamples, dilutions, and plates. Bacteria in millions per gram of oven-dried soil were plotted against dates of sampling. Similarly, moisture in percentage and air temperature in degrees F. were plotted against the same dates. The results are shown in Fig. 1–A, B, and C. The graphs for bacteria and moisture appear to be similar. Both indicate gradual decreases until Sample 12. taken August 4, followed by abrupt increases, and later downward trends. That for air temperature is quite different, showing a slight downward trend after the middle of July. On the basis of averages at each sampling, shown in Table I, the data for the season on moisture in percentage and bacteria in millions per gram of oven-dried soil give a correlation coefficient of .7564 and a regression coefficient of .9264. A theoretical estimate of the number of bacteria at each time of sampling was calculated from the moisture readings

TABLE I

Bacteria, millions	Moisture, %	Temp., °F.	Time, days	Bacteria, mult. regr.	Deviations, $A - T$	Deviations + base
29.68	33.16	56.58	1	29.09	.59	18.10
24.81	30.99	56.87	4	27.06	−2.25	15.26
30.88	36.40	55.50	8	31.11	− .23	17.28
29.65	31.00	59.47	11	26.39	3.26	20.77
26.64	32.29	68.02	15	26.88	− .24	17.27
28.16	30.71	65.30	18	25.40	2.76	20.27
26.91	34.91	69.43	25	28.08	−1.17	16.34
21.80	26.88	72.97	32	20.89	.91	18.42
17.93	26.05	69.83	39	19.67	−1.74	15.77
15.81	24.65	70.30	43	18.17	−2.36	15.15
13.73	19.96	72.93	46	14.05	− .32	17.19
10.66	17.06	67.87	53	11.21	− .55	16.96
24.31	33.95	69.63	67	23.58	.73	18.24
23.33	32.98	61.17	74	22.37	.96	18.47
18.73	27.52	75.10	78	17.28	1.45	18.96
18.34	34.93	60.60	88	22.72	−4.38	13.13
15.40	30.24	57.17	92	18.65	−3.25	14.26
18.05	30.16	67.17	95	18.09	− .04	17.47
19.58	30.59	63.60	99	18.17	1.41	18.92
20.46	29.40	63.80	100	17.11	3.35	20.86
16.70	27.69	41.12	107	15.63	1.07	18.58
15.04	27.10	38.17	110	14.96	.08	17.59
466.60	648.62	1382.62	1205			
$\bar{x}$=21.21	29.48	62.84	54.77			17.51

The base is a theoretical estimate calculated from moisture at 25%, temperature at 65° F. and time at 55 days.

on the basis of the linear regression equation. Then, in order to have moisture at about the same level as in the graph for actual numbers of bacteria (1–A), the differences between actual and theoretical estimates of the number of bacteria were plotted as deviations from a theoretical estimate based on a moisture reading of 25%. The finding is presented in Fig. 1–D. It shows that a large portion of the variation in numbers of bacteria in the plot from time to time is associated with differences in the moisture content of the samples used. Further, the line representing theoretical estimates with the effects of variation in moisture removed shows a definite downward trend.

In order to determine the variation in theoretical estimates when the effect of a second independent variable is removed along with that of moisture, multiple regression was applied to bacteria in millions, moisture in percentage and air temperature in degrees F. A multiple correlation coefficient of .7713 was obtained. This was tested for significance by calculating the F value according to Goulden (2). The result is an F value of 13.95, as compared to 5.93 at the 1% level of significance for 2 and 19 degrees of freedom. Again, theoretical estimates were calculated. The multiple regression equation with values substituted from the data follows: Y = numbers of bacteria.

$$\text{Estimated } Y = -45.5132 + .9641\ (\%\ \text{moisture}) + .6094\ (\text{degrees F.})$$

In this case, differences between actual and theoretical estimates of the number of bacteria were plotted as deviations from a theoretical estimate based on moisture at 25% and temperature at 65° F. The result is shown in Fig. 1–E and appears quite different from that in 1–A and 1–D.

In order to remove the variance causing the downward trend, apparent also in 1–E, and to examine the residual when the effect of still another variable is removed, a new multiple regression was applied involving all the data in Table I. In this case the data include a factor changing in intensity with time along with those considered previously. This factor is designated by a number for each date of sampling representing the days from the beginning of the experiment. In this analysis the multiple correlation coefficient is .9393, yielding the highly significant F value of 44.93. Here the regression equation becomes: Y = numbers of bacteria.

$$\text{Estimated } Y = 3.6706 + .8085(\%\ \text{moisture}) - .0230(\text{deg. F.}) - .0886(\text{days})$$

As before, theoretical estimates were calculated; and the differences between actual and theoretical estimates plotted, in this case, as deviations from a theoretical estimate based on moisture at 25%, temperature at 65° F. and the intensity of the factor associated with days at 55. This is presented as 1–F, and shows only minor variations in numbers of bacteria in this fallow plot when the effects of these three factors are removed by multiple regression methods.

(c) Type of Relation Between Moisture and Numbers of Bacteria

When numbers of bacteria are plotted against moisture the relation appears to be logarithmic. However, when numbers of bacteria adjusted for the factor

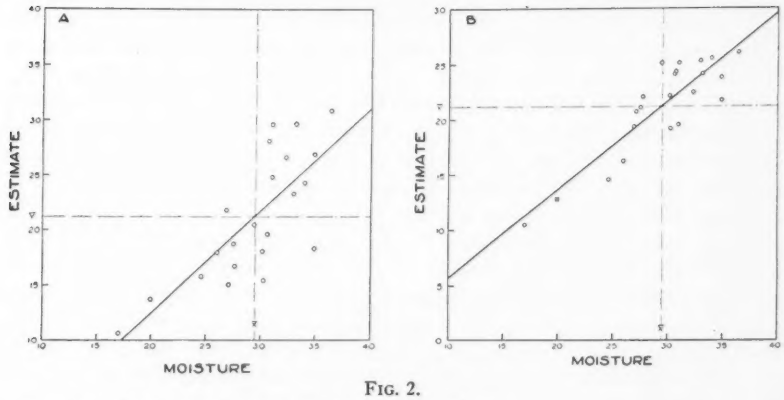

FIG. 2.

A...*X—moisture in percentage; Y—bacteria in millions.*

B...*X—moisture in percentage; Y—estimates of bacteria, representing differences between actual numbers and theoretical estimates calculated from the time factor by linear regression, plotted as deviations from a theoretical estimate based on the time factor at 55 days.*

Regression of bacteria on moisture in each case.

associated with time are plotted against the same moisture readings, the relation appears to be linear. This is shown in Fig. 2–B, where the fit of the linear regression line is much better than is the case in Fig. 2–A. In order to obtain further information on this problem, correlation coefficients were calculated from numbers of bacteria in the one case and from logarithms of the numbers in the other for the data of each of the illustrations. These are presented along with the variance for each set of numbers of bacteria. X = moisture in percentage; Y = bacteria in millions.

	2–A	2–B
Variance for Y	31.8683	17.7810
Correlation coefficient of X and Y	.7574	.8772
Correlation coefficient of X and log Y	.8029	.8898

In both cases the use of logarithms results in a slightly higher correlation coefficient. From the limited amount of data available it is not possible to draw a definite conclusion as to whether the relation is logarithmic. However, the results indicate only a slight improvement in the correlation obtained when logarithms are used on the data previously adjusted for the time factor, which also is correlated with numbers of bacteria.

Discussion

The value of the plate count as an indicator of the effects of various soil conditions or treatments depends, first, on a population sensitive to its environment, and second, on a technique capable of measuring small differences in

the population considered. A population sensitive enough to respond to differences in cropping or tillage would be expected to show the effects of marked changes in such a gross environmental factor as moisture. The finding that the refined technique used demonstrates a significant correlation between numbers of bacteria and moisture in replicate samples obtained from one plot at one time of sampling provides reason for encouragement. It indicates that the plate count technique has a place in the solution of the problems of the soil microbiologist.

While the air temperature data used in this study admittedly are of questionable value in relation to the number of bacteria in soil, the possible influence of soil temperature should be considered when the effect of another factor is under investigation. The soil temperature in a fallow plot undoubtedly would differ from that of a cropped plot in the same area. This effect might introduce an error in estimates of populations when cropped and fallow plots are involved in one experiment.

The downward trend in numbers of bacteria, which becomes more apparent when the effects of moisture and temperature are removed in the analysis, appears to indicate the presence of a factor that controls the supply of some essential food material. It should be noted that the plot under study was fallow. Consequently, there could be no effect attributable to growth of plants or the addition of organic matter. Further, the soil in the area is frozen from November to April. The mechanical effect of freezing and thawing of soil moisture may result in the disintegration of soil aggregates and the liberation of a fresh supply of the limiting nutrient in the spring.

When the environmental effects of moisture, temperature, and time are treated as constants, the data demonstrate that the number of bacteria varies within a rather narrow range. This finding, together with that concerning significant differences among estimates on samples obtained from one plot at one time when these environmental effects are not removed in the analysis, prompts a suspicion that the short-period fluctuations in bacterial populations referred to in the literature are the result of a combination of response to differences in the immediate environment from which the samples are drawn and lack of precision in the estimates.

In the light of these results, it is evident that an estimation of the effect of a treatment on the numbers of bacteria in soil includes the influence of changes in environment. These changes are not controllable under usual field conditions and may alter the result to such an extent as to obliterate differences produced by treatments, or introduce artificial ones. The influence of a treatment may be shown by a change in the effects of any or all of moisture, temperature, and time, as indicated by differences in averages, differences in the nature of regressions, or both: or it may be found in the residual after the effects of these environmental factors have been removed in the analysis.

Finally, multiple regression, accompanied by graphing of the results at various stages, may be applied to show differences not detectable by simpler analytical procedures. Additional information may be obtained from the

data, and an investigator may discover trends attributable to factors so interrelated as to be inseparable by other methods.

Acknowledgments

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THE INTERACTION OF HIGHER PLANTS AND SOIL MICRO-ORGANISMS

II. STUDY OF THE MICROBIAL POPULATION OF THE RHIZOSPHERE IN RELATION TO RESISTANCE OF PLANTS TO SOIL-BORNE DISEASES[1]

By M. I. Timonin[2]

Abstract

Comparative studies of flax varieties resistant and susceptible respectively to wilt, and of tobacco varieties resistant and susceptible to black root rot, showed higher numbers of micro-organisms in the rhizosphere of the susceptible than of corresponding resistant plants. Though plants of the same variety showed considerable variation in rhizosphere population under field and greenhouse conditions, the general trend remained the same. The abundance of micro-organisms in the rhizosphere of plants of the same variety grown in plots receiving different fertilizer treatment showed relatively little difference, even though the soils varied greatly in productivity.

Numbers of micro-organisms in the rhizosphere of flax were greater when the water content of the soil was maintained at 30%, than when held at 60%, of total moisture-holding capacity. However, the microbial population in the soil distant from the roots was lower in the drier soil.

Differential counts of fungi and actinomycetes indicated that the number of colonies developing from spores or conidia comprised a small proportion of the total count. Sporulation of fungi was more profuse in soil distant from the plant than in the rhizosphere.

The contact slide method indicated a greater number of micro-organisms in the rhizosphere than in soil distant from the roots and showed differences between the rhizosphere of resistant and susceptible varieties which agreed with results from the plating method.

Introduction

Results presented in the preceding paper (26) indicate that plants in the seedling stage (3 to 16 days after planting), grown under greenhouse conditions, are capable of stimulating the microbial activity about their roots, i.e., in the rhizosphere. Furthermore it was shown that different plant varieties stimulated the growth of different groups of soil micro-organisms. In the literature are found records concerning resistance or immunity to soil-borne diseases displayed by many varieties of cultivated plants, suggesting that certain varieties through the physiological function of their roots, involving the uptake of mineral nutrients from the soil solution, and the excretion of by-products of growth or by virtue of the chemical composition of the root cells, may control the activity of the pathogens in their rhizosphere. The present investigation was planned with the object of comparing the microbial activity in the rhizosphere of root-rot resistant and susceptible varieties of cultivated plants.

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Experimental

The procedure for the rhizosphere analysis was that previously described (26) except that different media were used. For bacteria, soil extract agar, prepared according to the method of Löhnis and without added energy material, was used. For fungi the medium, prepared from soil extract, contained, in addition to 0.2 gm. of dipotassium hydrogen phosphate, 0.1 gm. of yeast extract and 1.0 gm. of glucose per litre. Immediately prior to plating, the reaction was adjusted to pH 4.8 to 5.0.

In addition to total counts of fungi, the numbers of colonies developing from spores or conidia were also estimated by the "desiccation" method of McLennan (13). Counts of actinomycetes were made on a modified Czapek's sodium-nitrate–sucrose agar (10), in which sucrose was replaced by 10 ml. of glycerol, pH = 6.7 to 6.8.

The contact slide method described by Cholodny (2) and successfully applied by Hulpoi (11) was also used, the slides being stained with phenolic rose bengal (4) for 10 min. Other special procedures will be described under separate sections.

Rhizosphere Studies of Different Crop Plants in Soils Varying in Manurial Treatment

Preliminary studies were undertaken to note the influence of cropping as compared with manurial treatment on the density of the microbial population in the rhizosphere. Rhizosphere samples from oats, clover, and mangels were taken July 11 to 17, 1938, from plots of a four-year rotation (oats, clover,

TABLE I

DENSITY OF MICRO-ORGANISMS IN RHIZOSPHERE AS INFLUENCED BY MANURIAL TREATMENT OF THE SOIL

Rhizosphere of	Yield, 1938	Fungi, thousands	Actinomycetes, millions	Bacteria, millions
Plot N				
Oats	34.4 bu.	100.8	1.9	118.1
Mangels	3.63 tons	151.8	2.4	108.2
Clover	2.58 tons	805.8	1.7	254.8
Control (soil)*		74.3	2.6.	48.6
Plot X				
Oats	50 bu.	123.0	1.6	154.5
Clover	4.58 tons	819.0	1.9	286.8

Plot N = Received no fertilizer.
Plot X = 15 tons of farmyard manure applied under mangels.
** Samples obtained between the rows of mangels.*

timothy, mangels) receiving different fertilizer treatment. For 26 years Plot N had received no fertilizer, while Plot X received 15 tons of farmyard manure per acre, applied to mangels, and was in consequence much more productive than the former. With clover and oats it was virtually impossible to obtain control samples, i.e., free from roots.

The results, summarized in Table I, indicate that with the soils in question, which had become widely divergent in crop producing capacity, the type of crop was of much greater significance than the degree of fertility in determining the abundance of micro-organisms in the rhizosphere. Whereas plants of different kinds show marked variations in microbial numbers in the same soil, much lower fluctuations are noted in the rhizosphere of the same crop plant cultivated in soils that differ strikingly in fertility. The findings are in agreement with observations of Taylor and Lochhead (22) on the qualitative nature of the soil bacteria in the same plot series, which indicated that the relative incidence of the different bacterial groups was affected more by cropping than by fertilizer treatment.

The Activity of Micro-organisms as Influenced by Wilt Resistant and Susceptible Flax Varieties

A considerable amount of work has been done on the subject of resistance or immunity of plants to disease. Reviews of this literature have been published recently (14, 28). However, certain reports dealing with the resistance of cultivated plants to common root-rot diseases may be considered more closely.

Reynolds (15) found that extracts of flax plants resistant to wilt depressed the growth of *Fusarium lini* but not so the extracts from varieties of flax susceptible to this disease. He explained resistance as due to the presence of the glucoside linamarine. Dixon, Eckerson, and Link (5) explained the resistance of wheat seedlings to *Gibberella saubinetii* (Mont) Sacc. on the basis of the amount of pectins present in the cell wall. The fungus was able to infect varieties containing pectin in the cell wall, but not varieties devoid of the pectin. Rochlin (17) found that plants belonging to the Cruciferae containing glucosides such as sinigrine or gluconesturine were resistant to *Plasmodiophora brassicae*. Taubenhause and Ezekiel (20, 21), and Ezekiel *et al.* (7, 8, 9) reported that ether extracts from root juices of monocotyledons inhibited growth of *Phymatotrichum*, whereas those from roots of susceptible dicotyledons did not.

Conant (3), studying the resistance in tobacco varieties to *Thielavia basicola*, reported that resistant varieties were able to form a cork layer in the cambial growth that inhibited the spread of mycelium in the host tissue. Susceptible varieties, however, exhibited a lag in pericyclic division behind cambial development. Ullstrup (27), however, did not find any anatomical difference in the roots of susceptible or resistant varieties of China asters.

Field Experiments

To note whether plants of the same species, but known as varieties resistant and susceptible respectively to soil-borne diseases, would exercise different influences upon the micro-organisms in their rhizospheres, samples of healthy plants of the wilt-resistant "Bison" and wilt-susceptible "Novelty" were compared. Plants had been growing in sandy loam soil, and at the time of sampling were in the flowering stage.

The results presented in Table II which are based on the average of ten plants of each variety studied, indicate that the plants of the resistant variety harboured in the rhizosphere lower numbers of soil micro-organisms than the susceptible variety.

TABLE II

NUMBERS OF MICRO-ORGANISMS IN THE RHIZOSPHERE

Varieties	Type of plant	Fungi	Bacteria	Actinomycetes
Bison	Resistant	48.5	13.1	2.1
Novelty	Susceptible	75.6	18.1	3.0

Fungi in thousands, bacteria and actinomycetes in millions per gram of moisture free soil.

Greenhouse Experiments

Further studies were made with the same varieties of flax under greenhouse conditions. The soil, obtained from the surface layer of Plot X, was passed through a $\frac{1}{8}$-in. mesh sieve, and 200 gm. weighed out in each of 32 pots. To study the microbial population in the rhizosphere by the Rossi-Cholodny direct microscopic method, a sterile glass slide was introduced in a vertical position into the soil of each pot. Eight pots were sown with each variety of seed, four seeds to a pot, two seeds being in direct contact with the glass slide. Eight pots were kept unsown as controls. Seeds prior to planting were surface-sterilized in 1 : 1000 mercuric chloride solution for three minutes and then washed in five changes of sterile tap water.

Moisture content of the soil is considered an important factor in the development of the plant and also in the activity of micro-organisms. To study the influence of the moisture content of the soil on the activity of soil micro-organisms in the rhizosphere, the soil was adjusted in one-half of the pots to 60%, and in the second half to 30% of total moisture-holding capacity. These levels of saturation were maintained throughout the experiment by the "constant weight method" by the addition of distilled water.

The constant-capillary tension method was also used in this experiment. By this method water was continually supplied to the soil through the porous wall of the clay-cup "pressure cell". The details of the set-up and the cross section of the pressure cell are shown in Fig. 1.

The pressure cell, *a*, is a double-walled clay cup, with a porous inner wall that is in contact with the soil, and an air-proof outer wall. A rubber tube, *b*,

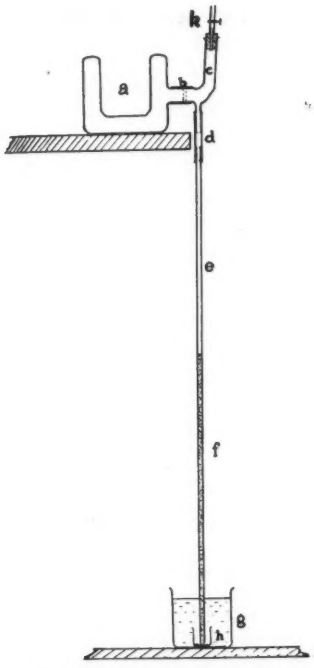

FIG. 1. *Diagram of set-up of pressure cell; explanation in the text.*

connects the pressure cell with the air trap, *c*. The air trap has a short glass tube projecting downwards, connected by means of a rubber tube, *d*, with the glass tube, *e*, 8 mm. inside diameter and 100 cm. long, and which may or may not contain a mercury column, *f*. The mercury column acts as a barostat. Glass tube *e* connects the pressure cell with the water jar, *g*. A 150 ml. glass beaker, *h*, supplies the necessary mercury to build up the mercury column and also collects it when pressure is released. When the inter-wall space of the pressure cell and the glass tube are filled with water and the rubber tube, *k*, is clamped, the capillary potential or the percentage of moisture of the soil in the pressure cell can be regulated by the height of the water and the mercury column (16).

The pressure cells were filled with air-dried soil that had been passed through a 40-mesh sieve. It was found by experiment that when the mercury column was raised in tube *e* to a height of 50 cm. the moisture content of the soil, calculated on the moisture free basis, was 14 to 15%. In the cells without the mercury column the moisture content of the soil was 27 to 28%. Two pressure cells were sown with each variety of seed, four surface sterilized seeds to the cell.

Samples for microbiological analysis were taken 15 days after planting. At the time of sampling, the glass slides were carefully removed from the soil, dried, and fixed, and the side that had been in contact with the seeds was stained with 1% phenolic rose bengal over a steam bath for 10 min.

Influence of Varieties of Flax upon the Soil Microflora

From the data presented in Table III, it is evident that the flax seedlings of both varieties supported a greater microbial population in the rhizosphere than in the soil distant from the roots. Furthermore, the data also clearly indicate the numerical difference in the microbial population in the rhizosphere of the two flax varieties. Bison, a variety resistant to wilt, supported in its rhizosphere a smaller number of micro-organisms studied, with the exception of actinomycetes, than did the susceptible variety, Novelty.

TABLE III

NUMBERS OF MICRO-ORGANISMS AS INFLUENCED BY THE MOISTURE CONTENT OF THE SOIL

Variety	Per cent of saturation	Sample from	Fungi	$\frac{R}{S}$ Ratio	Bacteria	$\frac{R}{S}$ Ratio	Actino-mycetes	$\frac{R}{S}$ Ratio
Constant weight method								
Bison (resistant)	60	R	146.2	1.39	341.5	2.49	11.9	1.3
		S	105.6		136.9		9.1	
	30	R	206.3	2.35	439.9	4.81	11.6	1.4
		S	87.8		91.4		8.1	
Novelty (susceptible)	60	R	244.1	2.29	1522.6	9.71	13.1	1.2
		S	106.8		156.7		11.0	
	30	R	500.5	6.84	2751.3	26.13	17.3	1.8
		S	73.1		105.3		9.4	
Moisture tension method								
Bison	60	R	183.2	1.34	732.4	5.21	13.9	1.5
		S	136.9		140.5		9.4	
	30	R	317.0	2.90	1103.4	10.90	13.1	1.5
		S	109.2		101.2		8.9	
Novelty	60	R	234.4	2.02	1051.9	6.90	12.6	1.4
		S	116.1		151.8		9.7	
	30	R	454.6	4.60	1439.0	12.01	13.6	1.5
		S	98.3		119.7		9.2	

R = *Rhizosphere.*
S = *Soil distant from the roots.*
$\frac{R}{S} = \frac{\text{Numbers in rhizosphere}}{\text{Numbers in the soil distant from the roots.}}$
Fungi in thousands, actinomycetes and bacteria in millions, per gram of moisture free soil.

The experiment was repeated three times; the numbers obtained varied considerably but the general trends of the density of microbial population in the rhizospheres of susceptible and resistant plants remained the same.

The marked numerical difference in the microbial accumulations in the rhizosphere of plants belonging by systematic classification to the same species may be attributed to differences in physiological function, particularly since the plants studied were free of disease and grown in disease-free soil. The results are of interest in view of the findings of Thom and Humfeld (24) who found increased numbers of micro-organisms on tobacco roots known to be infected or grown in infected soil, while fewer numbers were found on tobacco plants known to be resistant to root rot.

Influence of Moisture on the Density of Microbial Population in the Rhizosphere

The data in Table III also indicate that a lower moisture content of the soil tends to increase the density of microbial populations in the rhizosphere of susceptible as well as resistant varieties, but depresses the population in the soil distant from the roots. However, the marked differences in the density of organisms in the rhizospheres of resistant and susceptible varieties were more clearly indicated.

The constant weight method as applied for the maintenance of a certain degree of soil saturation is subject to criticism. For example, when the difference between the original weight of the pot and the weight obtained is made up by the addition of distilled water, the water tends to percolate more rapidly along the roots or by the walls of the container than in the soil itself, creating uneven distribution of moisture in the soil. To eliminate this uneven distribution of moisture, the "moisture tension method" was applied.

The experiment was started on December 12, 1938, and samples were analysed 15 days later. The greenhouse temperature during the experiment varied from 45 to 65° F., with relatively high air humidity. The results obtained with the plants grown under controlled conditions by the two methods are presented for comparison in the same table, and indicate the same general trend in the density of microbial population.

The general practice in studying microbial activity under controlled conditions is to adjust the moisture content of the soil to 50 to 60% of total moisture-holding capacity, which approximates the optimum requirement of the majority of cultivated plants. Reports by Sabinin and Minina (18), however, indicate that even in arid soil, which is practically sterile, the rhizosphere may show considerable microbial accumulations. Furthermore, Timonin (25) reported higher numbers of micro-organisms in horizons of Manitoba soils, with the moisture content below the wilting point, than in horizons of the same type of soil containing moisture available for plant growth. Results obtained by Eggleton (6) suggest that seasonal changes in moisture and temperature are not the direct causes of the seasonal changes

in numbers of bacteria in the soil, but control the growth of herbage which may be the factor responsible for the seasonal fluctuations of the soil population.

It is difficult to explain why the density of organisms in the rhizosphere is higher in the soil with 30% of total moisture-holding capacity than in that with 60%. There is no doubt, however, that the concentration of the soil solution in this experiment would be higher in the drier soil, and that this may influence the physiological function of the plant, and through it the root excretions that stimulate the growth of soil micro-organisms. Furthermore, the contact slides indicated that the formation of root hairs was more abundant in drier soil, giving a larger contact area of the roots per gram of rhizosphere soil. Consequently, a given weight of soil would contain a larger percentage of root excretion. In addition, numerous root hairs that function only for a few days would provide more easily decomposable organic matter. This, with higher concentrations of soil solution and root excretions, would presumably stimulate the activity of soil organisms, with resultant increase in numbers.

The rapidity of change in the density of the microbial population of the rhizosphere under changing moisture conditions was not studied, but the results indicate that further research in this line would probably throw some light on the seasonal fluctuations in numbers of organisms in cultivated soils.

State in Which Fungi are Recovered from Rhizosphere

The enumeration of fungi by the agar plating method provides no explanation of the precise origin of the colonies. They may have originated from spores or conidia, or from portions of mycelium. It is important, however, to know whether higher numbers in the rhizosphere are due to increased sporulation, which may be explained as a result of unfavourable conditions for growth of organisms, or to an increase in mycelial growth, which would indicate a favourable condition for fungal activity.

The data presented in Table IV indicate that colonies developing from spores or conidia represent only small fractions of the total count, namely 6 to 8%. Furthermore it is also evident that sporulation is greater in the soil distant from the roots than in the rhizosphere. The lower moisture content of the soil in the control soil tends to increase sporulation.

Several workers have presented evidence that actinomycetes are very resistant to drying. Thus Berestneff (1) was able to reisolate *A. violaceus* from inoculated ears of grain that had been stored for 10 years. Liske (12), however, stated that only conidia of saprophytic organisms were able to withstand desiccation for one and one-half years. However, the influence of desiccation *in vacuo* upon the mycelium or conidia of actinomycetes has not been studied in this investigation.

TABLE IV

NUMBERS OF ORGANISMS AS INFLUENCED BY DESICCATION

Per cent of soil saturation	Roots not desiccated		Roots desiccated		Per cent fungi colonies from spores	Per cent actinomycetes colonies from spores
	Fungi, thousands	Actinomycetes, millions	Fungi, thousands	Actinomycetes, thousands		
Bison						
60	68.8	14.1	7.7	329.1	11.0	2.3
30	185.3	16.7	10.4	381.2	5.7	2.3
Novelty						
60	166.5	10.8	13.0	445.9	7.8	4.1
30	207.5	14.6	19.3	1107.5	9.8	7.6
Soil distant from the roots						
60	45.4	6.9	5.3	552.3	11.7	8.0
30	32.1	8.4	5.7	492.2	17.8	5.9

Microbial Activity in the Rhizosphere as Recorded by Contact Slides

Examination of contact slides indicated noticeable differences in the abundance of the microbial accumulations in the rhizospheres and in soil distant from the roots. No distinct difference in the types of micro-organisms was observed in the rhizosphere of Bison and Novelty or in the control soil. The predominating forms of bacteria observed in all cases were short and long rods; however, coccoid forms and compact cyst-like aggregations similar to those reported by Starkey (19) were also noted.

On removal of the contact slide from the soil the rootlets in contact with the slide usually remain in the soil, leaving their imprints on the slide. These imprints are bordered with numerous root hairs most of which remained attached to the slide. It is extremely difficult to note the difference in abundance of micro-organisms about the root hairs of Bison and Novelty, (Figs. 2, 3, and 6, 7). There is, however, a noticeable difference in the density of organisms in the imprints of the rootlets of these varieties (Figs. 4, 5, and 8).

Starkey (19) noted an accumulation of bacterial colonies about fungus filaments and suggested that fungus mycelium was susceptible to bacterial attack. In this investigation, as shown in Figs. 2, 6, 9, and 12 the fungus mycelium is virtually free from bacteria. It may be that it was mere chance that fungus filaments did not come into contact with bacteria; however, the picture rather suggests the phenomenon of antagonism. It is true, however,

that the slides were kept in the soil for only 15 days, and it may be possible that after the death of mycelium the bacteria would be able to decompose it. Relatively few organisms developed on the slides that were kept in the control soil (without plants). (Figs. 10, 11, 12.)

The Activity of Micro-organisms as Influenced by Tobacco Varieties Resistant and Susceptible to Black Root Rot

To note whether the phenomenon of resistance in the case of flax varieties is also apparent with other plants, field samples of healthy tobacco plants, resistant and susceptible to black root rot, were obtained on August 2, 1938, from the same plot in adjacent rows.

Data presented in Table V indicate that the plants of the variety resistant to black root rot (R.H. 211) supported in their rhizosphere a lower number of micro-organisms than did those of the susceptible variety (C.H. 38). Data from the analysis of corn, samples of which were obtained on August 10, are

TABLE V

NUMBERS OF MICRO-ORGANISMS IN RHIZOSPHERE OF TOBACCO VARIETIES RESISTANT AND SUSCEPTIBLE TO BLACK ROOT ROT AND OF CORN

Variety	Type	Fungi	Actino-mycetes	Bacteria	Total number	Ratio of $\frac{\text{rhizosphere}}{\text{soil}}$
R.H. 211	Resistant	239.5	31.9	269.6	301.7	2.5
Control*		165.4	17.1	79.3	116.6	
C.H. 38	Susceptible	939.7	155.2	505.4	661.5	5.2
Control*		201.0	16.9	110.0	126.2	
Corn		775.1	15.4	389.2	405.8	12.4
Control*		91.3	4.5	28.2	32.8	

Fungi in thousands, actinomycetes and bacteria in millions per gram of moisture free soil.
* *Samples of control soil were obtained between the rows of plants.*

presented in the same table. Comparison of the rhizosphere-soil ratios indicate the pronounced ability of corn to stimulate microbial activity in the rhizosphere as compared with the tobacco varieties.

To verify the results obtained with plants grown under field conditions, plants of 13 tobacco varieties with different degrees of resistance to black root rot were grown under greenhouse conditions. The seeds were received from the Tobacco Division and their varietal resistance or susceptibility was unknown to the author. The seeds after surface sterilization were sown in rich greenhouse soil, and, at the fifth leaf stage, seedlings uniform in size were transplanted, one seedling per pot. The pots were filled with sandy

loam soil obtained from the surface layer of the experimental plot *N* in which tobacco had never been previously grown.

The first rhizosphere analysis of the seedlings was made 18 days after transplanting, and the second, 23 days later. The counts of micro-organisms were tabulated in order of density of microbial population in the rhizosphere. In this way three distinct groups were noted, namely, varieties with low, intermediate, and high microbial counts. When the varieties were thus classified the microbial counts were compared with the respective degree of resistance as determined by the Tobacco Division. Actually 10 out of 13 varieties were classified correctly by the microbiological method. A summary of the findings is presented in Table VI.

TABLE VI

NUMBERS OF MICRO-ORGANISMS IN RHIZOSPHERE OF TOBACCO PLANT VARIETIES WITH DIFFERENT DEGREES OF RESISTANCE TO BLACK ROOT ROT

Tobacco varieties	Fungi, thousands per gram			Actinomyces, millions per gram			Bacteria, millions per gram		
	1st Test	2nd Test	Average	1st Test	2nd Test	Average	1st Test	2nd Test	Average
Resistant—									
Havana C3X	161.6	143.9	152.8	0.8	4.4	2.6	55.7	201.2	128.5
Havana 211	57.7	*	28.9	1.0	6.4	3.7	134.7	43.6	89.2
Stand up	102.7	111.4	107.1	2.2	0.9	1.6	95.0	106.9	101.0
Stand up	112.6	41.5	77.1	2.4	7.4	4.9	96.0	174.4	135.2
Harrow Velvet	60.6	60.3	60.5	1.7	3.6	2.7	56.4	60.8	58.6
Harrow Velvet	53.4	91.6	72.5	2.0	3.3	2.7	56.3	64.0	60.2
Average	91.4	74.8	83.2	1.7	4.3	3.0	82.3	108.5	95.5
Intermediate in resistance—									
Connecticut Havana 38	97.6	96.1	96.9	1.6	4.6	3.1	83.7	93.0	88.4
Comstock Spanish	154.0	127.6	140.8	1.9	3.0	2.5	101.2	142.3	121.8
Kelley Burley	81.3	69.8	75.6	1.6	4.5	3.1	119.5	61.9	90.7
Judy Pride	73.4	87.8	80.6	2.8	4.2	3.5	117.2	106.3	111.8
Judy Price	59.2	84.8	72.0	2.2	3.1	2.7	149.5	106.0	127.8
Average	93.1	93.2	93.1	2.0	3.9	3.0	114.2	101.9	108.1
Susceptible—									
Greenwood	63.2	120.0	91.6	2.5	4.9	3.7	211.1	179.4	195.3
Greenwood	72.3	103.9	88.1	3.0	6.0	4.5	143.3	165.6	154.5
Average	67.8	112.0	90.4	2.8	5.5	4.1	177.2	172.5	174.9

* *No colonies developed on 1/1,000 dilution; numbers expressed per gram of moisture free soil.*

From the data it is quite apparent that bacteria are more sensitive to varietal differences in resistance than are fungi or actinomycetes. Fungi, especially, show considerable numerical variation. From the results obtained, however, it is felt that the adoption of certain improvements to standardize procedure, this method could be applied with advantage in aiding in the selection of varieties according to resistance to certain soil borne diseases.

Acknowledgment

The author wishes to express appreciation to Dr. A. G. Lochhead, Dominion Agricultural Bacteriologist, for advice during the experiments and for aid in the preparation of the manuscript. Thanks are also extended to Dr. W. G. McGregor, Cereal Division and Dr. N. MacRae, Tobacco Division, Experimental Farms Service, for co-operation throughout the course of the experiments.

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EXPLANATION OF PLATES

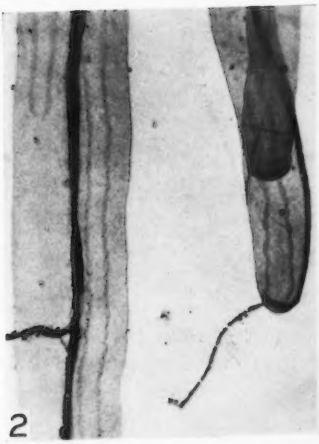
PLATE I—

FIGS. 2 – 7. *Microbial population in the rhizosphere of flax.* FIG. 2. *Root hair zone with few organisms observed. Septate branched fungus mycelium with few coccoid and short rod-shaped bacterial cells observed about fungus hyphae and root hairs. No sign of decomposition of root hairs and fungus hyphae. (Bison).* FIG. 3. *Root hair zone; same zone as in Fig. 2 but showing maximum microbial accumulation. Numerous chains of bacterial cells or actinomycete filaments and larger rod-shaped cells are predominating forms. No fungus hyphae observed. Root hairs possibly undergoing decomposition but it is also possible that they were injured by removal of slide from the soil. (Bison).* FIG. 4. *Imprint of rootlet; maximum microbial accumulation observed in this area. Actinomycete filaments and numerous rod-shaped and spherical bacterial cells. (Bison).* FIG. 5. *Same zone as in Fig. 4, showing minimum microbial accumulation. Actinomycete filaments and few deeply stained spherical and rod-shaped cells also visible. (Bison).* FIG. 6. *Root hair zone; minimum density of microorganisms observed in this area. Septate, branched, fungus hyphae surrounded by few rod-shaped bacterial cells. Colonies of rod-shaped and spherical bacterial cells but no fungus hyphae visible about the second root hair. No signs of decomposition of root hairs or fungus mycelium. (Novelty).* FIG. 7. *Root hair zone; same area as in Fig. 6 with maximum microbial accumulations. Rod-shaped bacterial cells predominant; deeply stained masses of bacterial cells. Protoplasm in the root hairs still visible. (Novelty).*

PLATE II—

FIGS 8 AND 9. *Microbial population in rhizosphere of flax.* FIG. 8. *Imprint of rootlet with maximum number of organisms. Actinomycete filaments and rod-shaped bacterial cells predominant; few spherical cells. (Novelty).* FIG. 9. *The fungus hyphae just outside the area shown in Fig. 8. No bacterial cells observed about fungus hyphae. (Novelty).*

FIGS 10 – 12. *Microbial population in the soil (control) without plants.* FIG. 10. *Maximum density of organisms observed. Colonies of long rod-shaped bacteria.* FIG. 11. *Minimum density observed on the same slide as in Fig. 10. Actinomycete filaments and few spherical cells.* FIG. 12. *Fungus hyphae also observed on the same slide, but no bacterial colonies developed about them.*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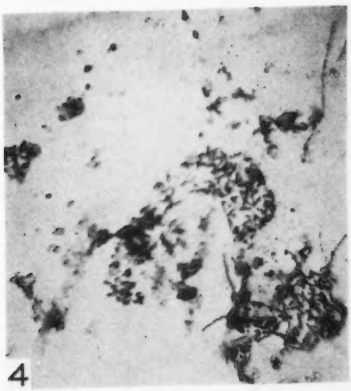
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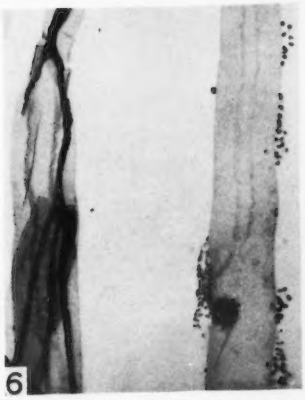
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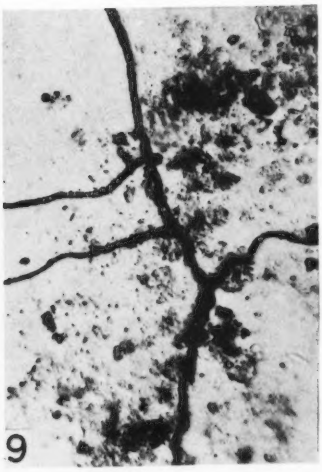
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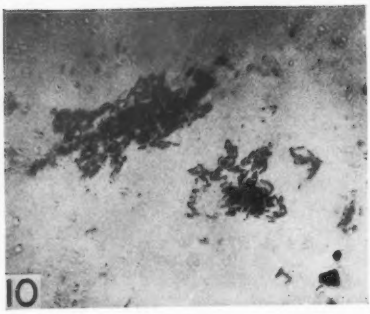
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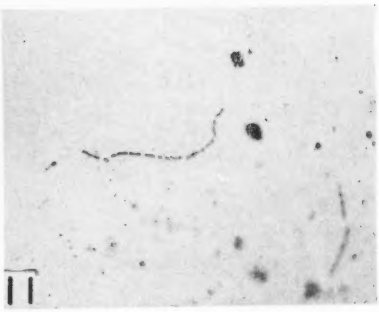
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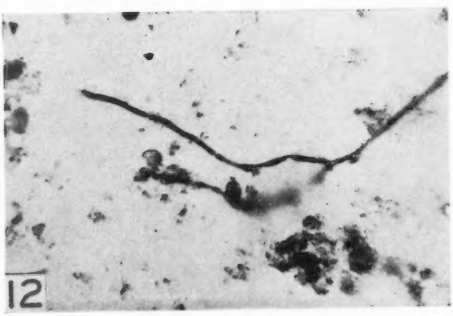
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RESPONSES OF PLANT CUTTINGS TO TREATMENT WITH NAPHTHYL ACIDS AND THEIR POTASSIUM SALTS IN A TALC CARRIER[1]

By N. H. Grace[2]

Abstract

Cuttings of one herbaceous and of four dormant woody plants were treated with a series of talc dusts containing indolylacetic acid and the acids and potassium salts of several members of the naphthyl series of root growth stimulating chemicals. The higher members of the series compared favourably with naphthylacetic acid in respect to most of the responses considered, though there were indications that naphthylacetic acid or its salt had a greater effect on the number of roots per rooted cutting. Naphthylacetic and 1-γ-naphthylbutyric members of the series were equally effective as acids or salts; however, a mixture of the isomeric 1- and 2-γ-naphthylbutyric acids was more active than the corresponding mixture of salts. Conversely, potassium naphthylhexoate appeared to have greater activity than the acid. The results suggest that pure naphthylbutyric acid, the isomeric mixture of acids, and potassium naphthylhexoate are virtually as effective as the recognized plant growth stimulating chemicals, indolyl- and naphthylacetic acids.

A noteworthy feature of the results was the beneficial effect of treatment with talc alone. The promotion of new growth and rooting of dormant cuttings were of particular interest, though most of the other criteria studied also indicated beneficial responses.

An earlier communication has shown that all the naphthyl acids from the acetic to the hexoic have a measure of physiological activity as determined by the rooting responses of solution-treated plant stem cuttings (4). The outstanding feature of the results was the activity of those members of the series with an even number of carbon atoms in the side chain. It was suggested that the higher members of the series might be particularly suited to the treatment of cuttings as they effected excellent rooting with, apparently, somewhat less danger of shock from overdosage than has been noted with the acetic member of the series. The present paper describes five further experiments in which cuttings of different plants were treated with talc dusts containing 1-naphthylacetic, 1-γ-naphthylbutyric, 1- and 2-γ-naphthylbutyric, and ε-(1-naphthyl)-hexoic acids. On this occasion, the series of dust treatments included the potassium salts of the naphthyl acids, as it was of interest to ascertain whether the relatively water soluble potassium salts were more active than the sparingly soluble long chain acids. The mixture of naphthylbutyric isomers was considered of particular interest from the practical point of view, since the mixture of the isomers is prepared more readily than the 1-γ-naphthylbutyric acid.

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Experimental

A series of 10 dusts was prepared, comprising talc and talc containing 1000 p.p.m. (parts of chemical per million parts of talc by weight) of the acids, and the potassium salts of the four naphthyl acids in amounts to provide the equivalent of 1000 p.p.m. of the acid concerned. All the dusts, including talc only, were subjected to a grinding mix operation for about 15 hr. (3). These 10 dusts, along with a control that contained no dust, provided a series of 11 treatments.

Each of the five experiments involved 352 cuttings divided into four replicated groups of eight cuttings* for each of the 11 treatments. These groups were planted in random order in the bench according to the principles of experimental design. Cuttings were sprayed with water prior to dusting to ensure uniform adhesion of a quantity of dust (15). They were then planted in brown sand in propagation frames equipped with electrical bottom heat cables. Sand temperature was maintained around 72° F., while that of the greenhouse room ranged around 65° F.

In the first four experiments, dormant woody cuttings were planted November 13 and 14, 1939. *Lonicera tartarica* L. cuttings were removed 48 days later, while those of *Sambucus canadensis* L. and *Deutzia scabra* Thunb. were removed 65 days after planting. *Hydrangea arborescens* L. cuttings were removed 78 days after planting. Cuttings of one herbaceous plant, *Iresine Lindeni* Lem., were planted February 22 and removed 30 days later. For the dormant cuttings, record was made of the number of cuttings rooted, the number showing new growth, and the number and length of roots per rooted cutting, except in the case of *Deutzia* of which, owing to a large number of roots, the length of root mass was determined instead of the root length (7). Further, where root length per rooted cutting was determined, the mean root length was calculated. In each case the weight of new growth per cutting with new growth was determined. Fresh root weights per group of eight cuttings were obtained for both *Hydrangea* and *Deutzia* cuttings. Owing to considerable secondary root development, in the case of cuttings of the latter plant, where root lengths per rooted cutting were not determined, fresh root weights per rooted cutting also were considered. Counts of the number of callused cuttings were made for *Lonicera* only, this count including all callused cuttings both with and without roots. Examination of the *Iresine* cuttings yielded data on the number of cuttings dead and rooted, the number of roots per rooted cutting, the length of root mass (7), the fresh root weight expressed in terms of root weight per rooted cutting and, finally, the fresh weight of the surviving cuttings after the roots were removed.

All data were subjected to analyses of variance. Data for all counts of numbers of cuttings were subjected to the inverse sine transformation prior to statistical analysis (1). In the experiment with *Lonicera* the rooting of the untreated and talc treated control groups was poor and the meagre data

* *The cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.*

for the number and length of roots and the mean root length were not suited to analyses of variance. Statistical analysis of these three responses was confined to the remaining nine treatments in this experiment.

Results

A table of data is presented for each of the five experiments but the details of the analyses of variance are not given. The necessary differences between individual treatment means have been calculated for all responses showing some significant treatment effects.

Lonicera tartarica

Data for the responses of *Lonicera* cuttings are given in Table I. None of the untreated cuttings rooted; rooting of the talc treated controls was below the mean of all hormone treatments and there were no other significant treatment effects. All the talc-treated cuttings (with and without hormone chemical) gave a greater number of callused cuttings than the untreated control group; none of the results with chemical treatments differed significantly from those with talc alone. Indolylacetic acid treatment produced fewer roots than the average for all naphthyl treatments. Both the hexoic acid and its salt gave fewer roots than the other naphthyl compounds, which did not differ among themselves in the effects produced. The average effect of naphthyl acid or salt treatment was to produce a greater length of root per rooted cutting than that resulting from indolylacetic acid application. Treatments, neither as a whole nor on partition, attained significance in respect to the mean root length. The number of cuttings showing new growth was increased in marked manner by talc treatment. Data for the weight of new growth per cutting with new growth have been included, but they failed to reveal any significant treatment effects. The data, on the whole, fail to show any average differences between naphthyl acids or salts, and the results for the mixture of isomeric butyric acids in no instance differ from those for the pure 1-γ-naphthylbutyric acid.

Sambucus canadensis

In Table II are given data for the responses of *Sambucus* cuttings. A substantial increase in the number of rooted cuttings was noted following treatment, but this was apparently attributable to the talc rather than to the phytohormone acids or salts. Similarly, talc treatment increased the number of roots per rooted cutting. The results for both the acids and salts of the isomeric mixture of butyric acids suggest a greater number of roots than that following application of the pure chemical, but the observed differences are not statistically significant. The length of root per rooted cutting was also increased by all the talc treatments. While the groups of acids and salts failed to differ on the average, the salts of the isomeric mixture of butyric acids excelled the hexoate in respect of root length. All 10 talc treatments produced a greater mean root length than the untreated group of cuttings. Similarly, all 10 talc treatments resulted in a substantial increase in the number

TABLE I

RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Untreated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ϵ-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ϵ-(1-naphthyl)-hexoic	
Number of cuttings rooted	Transformed data	0	20.7	37.7	39.5	45.0	49.3	35.3	56.3	44.3	46.8	33.9	19.0
	Per cent	0	21.9	37.5	40.6	50.0	56.3	34.4	68.8	50.0	53.1	31.3	
Number of cuttings callused	Transformed data	16.5	54.8	48.8	43.1	47.0	55.4	46.8	58.1	48.6	50.6	42.7	19.0
	Per cent	21.9	65.6	56.3	46.9	53.1	65.6	53.1	71.9	56.3	59.4	46.9	
Number of roots per rooted cutting		+	+	5.5	17.4	11.4	10.3	5.1	14.3	13.7	13.4	5.6	7.2
Length of root per rooted cutting, mm.		+	+	89	382	274	249	159	298	295	287	100	184
Mean root length, mm.		+	+	14.7	21.8	22.2	24.6	25.9	21.8	18.9	21.0	18.5	10.1
Number of cuttings with new growth	Transformed data	9.5	54.8	50.6	45.0	43.2	38.8	41.4	58.6	42.5	48.6	41.3	18.0
	Per cent	9.4	65.6	59.2	50.0	46.9	40.6	43.8	71.9	46.9	56.3	43.8	
Weight of new growth per cutting with new growth, gm.		+	0.31	0.40	0.28	0.39	0.39	0.39	0.41	0.55	0.37	0.38	

+ *Meagre data, not suited to analysis of variance.*

TABLE II

RESPONSES OF DORMANT *Sambucus canadensis* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Untreated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	
Number of cuttings rooted	Transformed data	43.2	64.7	64.7	69.3	65.6	62.7	65.6	65.6	70.2	60.3	56.6	16.0
	Per cent	46.9	81.3	81.3	87.5	78.1	78.1	78.1	78.1	84.4	75.0	68.8	
Number of roots per rooted cutting		5.1	8.4	9.0	11.1	7.9	9.3	8.7	9.9	8.2	10.7	6.9	3.2
Length of root per rooted cutting, mm.		187	384	380	467	363	406	366	392	344	446	316	120
Mean root length, mm.		30.5	46.6	42.2	41.6	46.1	42.7	41.6	39.5	41.8	42.1	45.3	7.9
Number of cuttings with new growth	Transformed data	48.8	84.8	72.2	79.7	72.2	75.0	75.0	72.2	75.4	72.2	75.4	22.1
	Per cent	56.3	96.9	87.5	93.8	87.5	87.5	87.5	87.5	87.5	87.5	87.5	
Weight of new growth per cutting with new growth, gm.		0.08	0.22	0.19	0.25	0.23	0.19	0.22	0.22	0.22	0.25	0.18	0.09

of cuttings developing new growth. The weight of new growth per cutting with new growth was increased from two to three times by talc treatment. There were no other significant treatment effects. The beneficial effects of talc treatment thus stand out as the main feature of the results as a whole. There were no average differences between the groups of acids and salts, and the effects of the isomeric naphthylbutyric acids did not differ significantly from those of the pure butyric acid.

Deutzia scabra

The responses of *Deutzia* are presented in Table III. These cuttings rooted well and few treatments gave less than 90% rooting. The rooting of the talc control group just failed to exceed that of the untreated cuttings, but an increase was suggested. Hormone treatments, on the average, suggested a further increase in rooting, though none of the treatments differed significantly from the talc control in this respect when considered individually. Treatment with talc alone had no effect on the number of roots per rooted cutting of this species. Addition of naphthylacetic and the isomeric butyric acids resulted in more roots per rooted cutting than the number obtained with talc only, and all the salts, excepting those of the mixture of isomeric acids, yielded more roots than the talc control. An interesting reversal effect was noted. Treatment with salts of the isomeric butyric acids resulted in the production of fewer roots than did treatment with the acids, but the salt of hexoic acid produced more roots than the acid. The length of root mass was greater following application of the isomeric butyric acids than after the hexoic acid treatment. The latter reduced length of root mass below that of the talc control.

The number of cuttings showing new growth was, on the average, increased by talc treatment. None of the naphthyl treatments differed among themselves in this respect. Most of the naphthyl treatments resulted in lower weights of new growth than that following application of indolylacetic acid, which gave the greatest weight. Fresh root weight per group of eight cuttings appeared to be diminished following treatment with talc alone. However, a number of the naphthyl treatments resulted in weights exceeding those obtained following talc only. The isomeric butyric acids and the potassium salt of naphthylacetic exceeded the untreated control in respect to root weight, while the hexoic acid was below it. While there was no difference on the average between the results from acids and salts, certain differences between the chemicals were highly significant. Both acids and salts of the acetic and pure butyric members of the naphthyl series resulted in essentially the same weight of roots. Data for the weight of roots per group of eight cuttings and weight of root per rooted cutting indicated essentially the same type of reversal effect already discussed for the number of roots per rooted cutting. Further, talc treatment resulted in a lower weight of root per rooted cutting than occurred with the untreated group of cuttings.

TABLE III

RESPONSES OF DORMANT *Deutzia scabra* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Untreated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	
Number of cuttings rooted	Transformed data	62.7	77.3	82.5	77.3	79.7	79.7	69.3	90.0	65.9	77.3	67.0	14.7
	Per cent	78.1	90.6	93.8	90.6	93.8	93.8	87.5	100.0	78.1	90.6	84.4	
Number of roots per rooted cutting		19.3	17.3	21.0	28.0	24.8	28.3	15.5	30.0	25.0	18.8	26.8	7.7
Length of root mass, mm.		66.0	57.3	56.8	52.5	56.3	62.5	45.0	51.0	62.5	58.3	58.3	12.0
Number of cuttings with new growth	Transformed data	58.6	72.2	84.8	69.8	79.7	67.9	62.3	73.1	67.9	67.9	79.7	18.5
	Per cent	71.9	87.5	96.9	84.4	93.8	81.3	78.1	84.4	81.3	81.3	93.8	
Weight of new growth per cutting with new growth, gm.		0.51	0.49	0.61	0.32	0.44	0.37	0.49	0.37	0.42	0.32	0.42	0.18
Fresh root weight, gm.		3.5	2.5	3.4	4.6	4.0	4.9	1.6	5.2	3.3	2.8	4.2	1.3
Average fresh root weight per rooted cutting, centigrams		55	36	45	64	53	67	23	65	56	39	61	19.0

The results, as a whole, indicate comparatively little effect from talc treatment. While there were no differences, on the average, between the response to naphthyl acids and salts taken as groups, there were marked differences between the behaviour of acids and salts of individuals. This difference was brought out by the drop in activity from the isomeric butyric acids to their salts and the increased activity of the hexoate as compared with hexoic acid.

Hydrangea arborescens

In Table IV are given data for the responses of *Hydrangea* cuttings. In the main, the responses of these cuttings disclosed treatment effects similar to those discussed in detail in the preceding three tables. Talc treatment had a marked beneficial effect on most of the responses. While there were no significant differences in the behaviour of the naphthyl acids or salts as groups, there were marked differences between the acids and salts of the last two members of the series. This reversal effect was closely similar to that discussed in detail for several of the responses of *Deutzia* cuttings (Table III). The isomeric mixture of butyric acids appeared rather more active than the corresponding mixture of salts, whereas naphthylhexoate was more active than the hexoic acid.

Iresine Lindeni

Data for the responses of *Iresine* cuttings are given in Table V. In general, treatment effects disclosed by the responses of these herbaceous cuttings were similar to those shown by dormant cuttings of the four woody plants. Beneficial effects of talc treatment were indicated, particularly in relation to reduced mortality. Injurious effects, as shown by increased mortality and reduced rooting, following treatment with 1000 p.p.m. indolylacetic acid, were however in contrast to the behaviour of the other plants considered. The number of roots per rooted cutting following naphthylacetic treatment was above that obtained with all other treatments to an extent not shown by any of the other plants. There were no differences between the groups of acids and salts but marked differences between the acids and salts of individual members of the naphthyl series in respect of length of root mass. The behaviour of the isomeric mixture of butyric acids and their salts and the hexoic acid and its salt was in agreement with the reversal effect mentioned in the case of *Deutzia* and *Hydrangea* cuttings, since physiological activity of the chemical appears to vary inversely with the length of root mass. Further, 1-γ-naphthylbutyric acid and its salt differed in respect of length of root mass. This was the only instance of any difference in effect of acid and salt of this member of the series. On the average, the naphthyl treatments resulted in a greater weight of root per rooted cutting than the untreated, talc or indolylacetic acid treated groups. Several of the naphthyl treatments were above indolylacetic acid in respect to root weight.

TABLE IV

RESPONSES OF DORMANT *Hydrangea arborescens* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Untreated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ϵ-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ϵ-(1-naphthyl)-hexoic	
Number of cuttings rooted	Transformed data	33.8	69.8	54.3	60.4	62.3	53.0	54.5	52.4	73.6	50.7	75.0	23.7
	Per cent	37.5	84.4	65.6	75.0	71.9	62.5	59.4	62.5	84.4	59.4	87.5	
Number of roots per rooted cutting		3.8	6.1	6.2	11.3	11.9	13.2	7.7	9.6	11.4	8.3	10.0	4.3
Length of roots per rooted cutting, mm.		81	144	130	191	200	244	139	138	229	140	184	81
Mean root length, mm.		15.8	24.4	21.8	17.4	17.0	19.1	19.0	14.4	19.7	16.4	18.6	7.4
Number of cuttings with new growth	Transformed data	43.4	72.2	52.2	59.9	66.6	54.8	63.2	52.4	73.6	52.4	72.2	23.1
	Per cent	53.1	87.5	62.5	68.8	78.1	65.6	68.8	62.5	84.4	62.5	87.5	
Weight of new growth per cutting with new growth, gm.		0.16	0.25	0.25	0.15	0.17	0.17	0.19	0.18	0.18	0.18	0.20	0.10
Fresh root weight, gm.		0.17	0.56	0.38	0.63	0.61	0.60	0.33	0.37	0.80	0.34	0.70	0.35

TABLE V

RESPONSES OF *Iresine Lindeni* CUTTINGS TREATED WITH TALC DUSTS CONTAINING PLANT HORMONE ACIDS AND POTASSIUM SALTS

Data are means for four groups of eight cuttings

		Controls		Treated with talc containing 1000 p.p.m. of									Necessary difference, 5% level
				Acids					Potassium salts of				
		Untreated	Talc treated	Indolyl-acetic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	1-naphthyl-acetic	1-γ-naphthyl-butyric	1 and 2-γ-naphthyl-butyric	ε-(1-naphthyl)-hexoic	
Number of cuttings dead	Transformed data	35.6	5.2	33.5	5.2	19.8	0	5.2	20.2	5.2	5.2	7.5	19.0
	Per cent	40.6	3.1	31.3	3.1	15.6	0	3.1	15.6	3.1	3.1	6.3	
Number of cuttings rooted	Transformed data	49.4	84.8	56.5	84.8	70.2	90.0	84.8	69.8	84.8	84.8	82.5	16.9
	Per cent	56.3	96.9	68.8	96.9	84.4	100.0	96.9	84.4	96.9	96.9	93.8	
Number of roots per rooted cutting		16.3	19.3	23.0	33.8	25.8	22.8	22.8	33.3	26.5	24.5	24.3	5.4
Length of root mass, mm.		22.3	21.5	17.5	14.8	24.3	22.5	26.5	16.3	20.5	23.3	22.8	3.7
Fresh root weight per rooted cutting, centigrams		1.7	1.9	1.4	1.6	2.2	2.3	2.3	2.0	2.3	2.2	2.0	0.8

Discussion

It is known that the higher members of the naphthyl series are active when used in solutions. The experiments just described show that when applied in a talc carrier, they compared favourably with naphthylacetic acid or its salt in respect to the number of cuttings rooted. This fact is of considerable interest since the effectiveness and scope of the dust carrier method of applying root growth promoting chemicals has been indicated by recent developments in vegetative propagation (3, 5, 8–15). Most of the other responses considered indicated that the higher members of the naphthyl series had physiological activity of the same order as naphthylacetic acid. On the whole, the activity of the isomeric mixture of butyric acids and the hexoic member of the series compared favourably with that of the other two members under test. However, the hexoic member of the series appeared, in several instances, to have somewhat less effect on the number and length of roots. This fact may be related to the use of identical weights of the various chemicals, since equal proportions in the dust provide fewer of the larger hexoic molecules.

In none of the five experiments was there any significant difference between the average effect on any response of the groups of naphthyl acids or salts. However, in several experiments there were substantial differences between the acids and salts of the isomeric butyric and the hexoic members of the naphthyl series. There was a tendency for the isomeric acids and the potassium salt of naphthylhexoic acid to be more active than the corresponding mixture of salts or the hexoic acid. It would appear justifiable to attribute this behaviour of the isomeric mixture to the presence of 2-γ-naphthylbutyric acid. The reduced activity of its salt would seem to be related to greater solubility or transport considerations. The activity of potassium naphthylhexoate would also appear to be related to solubility or transport considerations. These results would not suggest that salts of growth stimulating acids are always to be preferred to the acids (16).

Reference may be made to one point that has frequently been stressed by the author's experiments on propagation. This relates to the variability in different parts of the propagation frame which, in conjunction with the natural biological variability, renders it most important to plan experiments in a manner that will permit of some test of significance of the results. A case in point is noted in Table I. The weight of new growth per cutting with new growth varied from a low of 0.28 gm. after naphthylacetic acid treatment, to a high of 0.55 gm. after application of potassium 1-γ-naphthylybutyrate. Despite this approximately 100% difference in weight, statistical analysis showed that the variability of the data was too great to demonstrate any significant effects. In consequence any attempt to discern treatment effects from the means of single small groups of cuttings without replication seems a most dubious procedure.

A rather striking feature of the results was the beneficial effect of the talc treatment. Beneficial effects from treatment with dusts have been recognized

previously (5, 6, 11, 12, 14, 15). Not only root development but even the initiation of new growth by dormant cuttings was substantially increased by talc treatment. It appears likely that water relations do account for a considerable part of the beneficial effect (11). However, it is also probable that availability of certain mineral elements may be involved, since nutrient treatments have been shown to affect the rooting of cuttings (2, 6, 10, 15). This possibility would seem to be indicated by data in Tables II and IV, which show that talc treatment increased the weight of new growth per cutting with new growth. There is, of course, the possibility that an adsorbent such as talc could retain natural plant hormones that might be leached from the open end of the cuttings. Complete elucidation of the mechanism of the action of talc on cuttings awaits further experimental study. However, the facts available to date indicate that the carrier dust method of treating plant cuttings has numerous advantageous features.

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THE GEOGRAPHICAL DISTRIBUTION OF THE GENUS *GYMNOSPORANGIUM*[1]

By Ivan H. Crowell[2]

Abstract

This study of geographical distribution shows that species of the genus *Gymnosporangium* are found in the northern hemisphere only and occur most abundantly in the temperate portion. Each of the three major continents contains a distinctive *Gymnosporangium* flora and, with the exception of three species called the "tricontinental species", species occur naturally in one continent only. The genus contains about 48 species; 33 occur in North America, 15 in Asia and 6 in Europe (including the three tricontinental species in each case). Explanations of the types of geographical distribution of the North American species are given under four categories: (i) species that occupy all potential territory covered by the coincident ranges of their alternate hosts, (ii) species that are confined by the range of their "primary" telial host, (iii) localized species that are confined within a portion of the coincident ranges of their alternate host, and (iv) widely distributed species that are not limited in their range by either alternate host group.

Introduction

The phytopathological and mycological literature is replete with incidental statements on the geographical distribution of fungi, yet few authors have dealt comprehensively with this subject. In contrast to the geographical distribution of higher plants, that of fungi is limited by the distribution of their living or dead substrata. Such limitations are particularly true of parasitic fungi that can exist on living substrata only. Of especial interest is the geographical distribution of opsis-form rusts, such as species of *Gymnosporangium*, for these obligate parasites, lacking the uredial or repeating-spore stage, have no means of perpetuating themselves beyond the coinciding ranges of the two groups of alternate host plants. It might be expected that species of *Gymnosporangium* would occupy completely territory covered by the coinciding ranges of their alternate hosts. Such, however, is seldom the case.

The genus *Gymnosporangium* is composed of about 48 species and is confined almost entirely to the temperate portion of the northern hemisphere. This region, especially North America and Europe, is probably more intensively explored and its microflora more thoroughly known than is the case with any other. Since the fruiting structures of species of *Gymnosporangium* are quite conspicuous, it seems fair to assume that the numerous records in the literature and on collections deposited in herbaria afford comprehensive (though obviously incomplete) material on which to base geographical studies.

The present account of the geographical distribution of the genus *Gymnosporangium* is offered after the writer's examination, during the past seven years, of approximately three thousand records.

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The pertinent literature of North America, Asia, and Europe was carefully read and notes were taken of reports of the occurrence of all species of the genus *Gymnosporangium* on all hosts. These notes were augmented by examination of specimens from several herbaria in the United States, Canada, and Europe. Furthermore, several co-operators sent lists of the species of *Gymnosporangium* in their herbaria. From these notes and the writer's own observations, host lists were compiled for each species. Maps were drawn showing the station for each report of the rust wherever possible—a dot representing the station. Superimposed on these maps were representations of the geographical ranges of *(a)* the O–I hosts indicated by vertical lines, and *(b)* the III hosts, indicated by horizontal lines. Rehder's Manual (1927) was the principal source for information on taxonomy and distribution of hosts, but several other taxonomic works were also consulted.

Upon analysing the maps of distribution of the species of *Gymnosporangium* it was found that three major geographical regions were clearly discernible. These geographical regions were contained one within each of the continents of North America, Asia, and Europe (including the adjacent portion of northern Africa). In the North American region, two minor geographical areas were also evident. These were *(a)* the eastern region, extending from the Atlantic ocean to the foothills of the Rocky Mountains, and *(b)* the western region, extending from the foothills of the Rocky Mountains westward to the Pacific Coast.

Three species of *Gymnosporangium* occur on all continents of the northern hemisphere and hence did not find a definite place in the discussion of species of any one region. The three species are dealt with separately as the "Tricontinental Species". Discussion of the geographical distribution of other species of *Gymnosporangium* are presented according to the scheme of major and minor geographical areas.

The Tricontinental Species

Three species of *Gymnosporangium* are found throughout the northern hemisphere. They are *(a) G. aurantiacum* Chev., *(b) G. clavariaeforme* (Jacq.) DC., [*G. gracile* Pat., *G. orientale* Syd., *G. oxycedri* Bres.], and *(c) G. juniperinum* (L.) Mart., [*G. amelanchieris* Fisch., *G. ariae-tremelloides* (A. Br.) v. Tub.]. For the purposes of clarity in this account, marginal or inadequately differentiated species have been included as synonyms of the three broadly conceived species *G. aurantiacum*, *G. clavariaeforme*, and *G. juniperinum*. This procedure follows closely that of Eriksson (8). Recognition of forms of doubtful specific rank would have necessitated treatment of small, closely related entities, most of which seem to be of local distribution on a few hosts.

The tricontinental species have several characteristics in common apart from their wide distribution. They are most abundant in and most closely related to the European *Gymnosporangium* flora. Their principal telial hosts are species of *Juniperus* in the section Oxycedrus, chiefly *J. communis*.

Gymnosporangium clavariaeforme and *G. juniperinum* have been reported to attack species in the section Sabina, but they do not seem to do so frequently.

Each of the tricontinental species occurs over the greater part of Europe and adjacent north Africa. In other geographical regions each species is less abundant and stations are more widely scattered, though hosts for them seem to have an unbroken distribution throughout the greater part of the temperate portion of the northern hemisphere.

Details of the geographical distribution of these species are shown in Figs. 1, 2, and 3 respectively. Stations for the three tricontinental species are brought together in Fig. 4. Their distribution in Europe is essentially like that of strictly European species. Their distribution in North America is scattered and broken. The conspicuous gap in distribution from Europe to coastal Asia probably represents a lack of knowledge of the fungi of this vast, little explored region rather than an absence of these species of *Gymnosporangium*.

Species of the Eastern North American Region

A. Endemic Species—

1. *G. bermudianum*
2. *G. biseptatum*
3. *G. clavipes*
4. *G. corniculans*
5. *G. davisii*
6. *G. ellisii*
7. *G. exiguum*
8. *G. externum*
9. *G. floriforme*
10. *G. globosum*
11. *G. hyalinum*
12. *G. juniperi-virginianae*
13. *G. nidus-avis*
14. *G. trachysorum*
15. *G. transformans*

B. Tricontinental Species—

16. *G. aurantiacum*
17. *G. clavariaeforme*

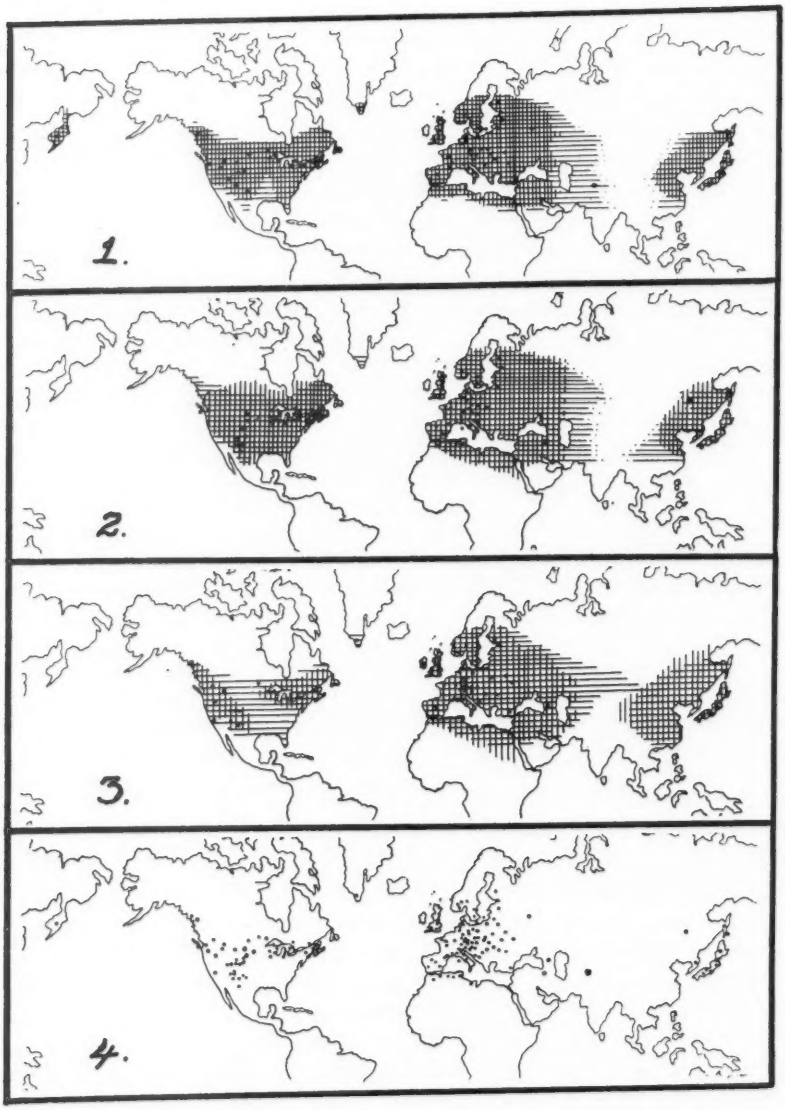
C. Exotic Species—

18. *G. haraeanum*
19. *G. japonicum*

The eastern North American region contains one of the richest and most diversified *Gymnosporangium* flora of the world. This wealth of material is significant in several respects. Nineteen of the 48 species discussed are recorded from this region; of these, 15 are endemic, 2 are exotic (native to Asia), and 2 of the tricontinental species are considered to be indigenous.

The greatest concentration of species of *Gymnosporangium* is found along the Atlantic coastal area of the eastern region, and the abundance of stations diminishes toward its western boundary. With the exception of a few stations for *G. clavipes* and *G. juniperi-virginianae* in the western region of North America, all other endemic species are strictly localized within the eastern region, none of them ranging over the maximum territory occupied by the coincident ranges of their alternate hosts.

Four species are generally distributed in eastern North America. They are *G. juniperi-virginianae* Schw., *G. clavipes* C. & P., *G. globosum* Farl., and



FIGS. 1 – 4. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for the rust.* FIG. 1. *G. aurantiacum.* FIG. 2. *G. clavariaeforme.* FIG. 3. *G. juniperinum.* FIG. 4. *The tricontinental species.*

G. nidus-avis Thax.* (Figs. 5, 6, 7, and 8.) It will be seen that although their distribution is wide, none of these species occupies all potential territory; *G. juniperi-virginianae* seems to make the nearest approach to this maximum, but a consideration of the following will show this to be far from true. Pomaceous hosts for this species include all native species and many orchard varieties of *Malus*. The combined ranges of these plants is essentially transcontinental in temperate North America. So also are the combined ranges of the telial hosts *Juniperus virginiana* L., *J. scopulorum* Sarg., and *J. horizontalis* Moench. The fungus, however, is confined almost entirely within coincidental ranges of native species of *Malus* and *J. virginiana* in eastern North America.

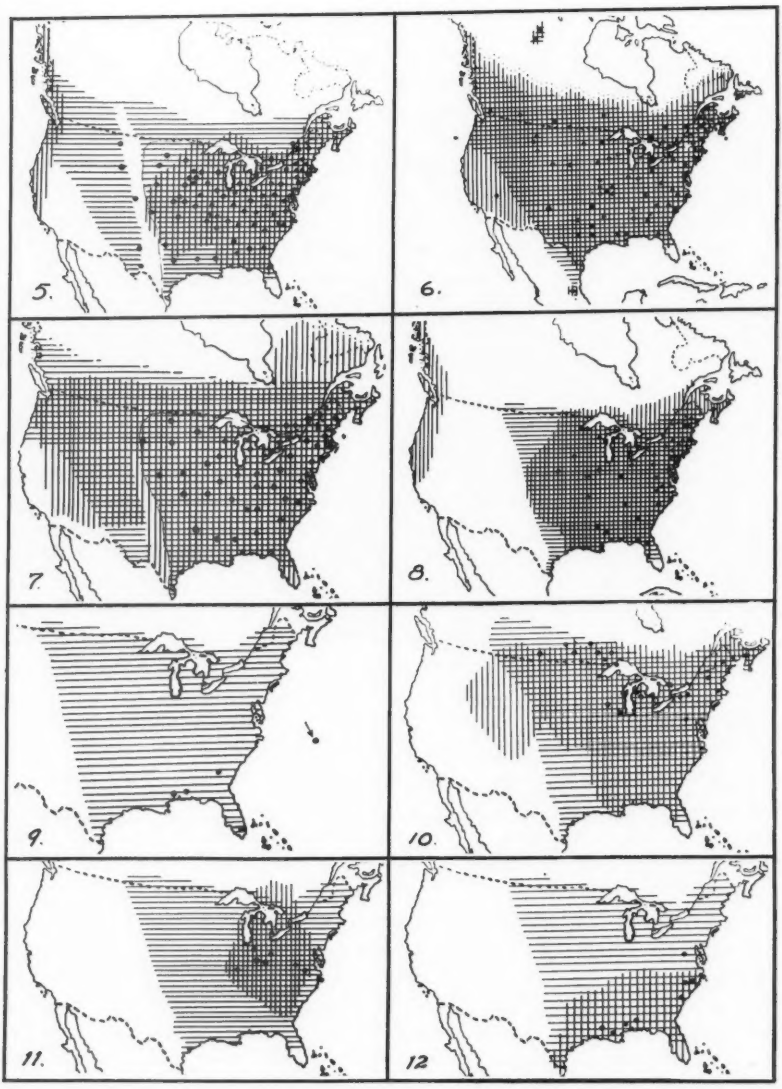
In Fig. 5 are shown four stations in the Rocky Mountains beyond the range just mentioned. These stations occurred on *J. virginiana* and *J. scopulorum*. In two stations in New Mexico that were checked by the author, the rust was found on hosts imported for ornamental purposes. No evidence of the rust could be found after the galls produced one crop of spores.

G. clavipes is a species of exceptional interest to the problem at hand because of its great longitudinal and latitudinal range. The species extends from Lake Athabasca, Alberta, to Mexico City, Mexico, and from the Atlantic Ocean almost to the Pacific. *G. clavipes* is the only species to parasitize consistently telial hosts in two sections of the genus *Juniperus*, in particular *J. virginiana* in the section Sabina and *J. communis* in the section Oxycedrus. Its pomaceous and *Juniperus* hosts are associated over the greater part of the northern hemisphere, yet this species is confined to North America and is predominately an eastern species. As stations for *G. clavipes* are being recorded from time to time in the western region, it appears that it may be actively extending its geographical range, a phenomenon not in evidence in the case of any other species of the genus.

Though alternate hosts for *G. globosum* are transcontinental in range, the rust is found in eastern North America almost entirely within the range of the telial host *J. virginiana*. With the exception of *Malus fusca* all other endemic hosts of *G. nidus-avis* extend over much of the area occupied by *J. virginiana*.

The four species discussed in the foregoing are probably the most intensively studied in the genus. In contrast to the wide distribution of these species, certain others are conspicuously limited in their geographical range. *G. bermudianum* (Farl.) Earle (Fig. 9) is an outstanding example. It is an autoecious species and hence has a potential range as great as the combined ranges of its hosts—*J. virginiana* L. and *J. barbadensis* L. The rust has been reported from relatively few widely separated stations in Bermuda and the Bahamas Islands and from a narrow strip of the mainland bordering the Gulf of Mexico. One station, more inland, is located in Georgia.

** On the basis of numerous cultural experiments and microscopic examinations, the author considers G. nidus-avis Thaxter (Bull. Conn. Exp. Sta. 107 : 3, 1891) and G. effusum Kern (Bull. N.Y. Bot. Gard. 7 : 459, 1911) to be synonymous.*



FIGS. 5 – 12. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for the rust.* FIG. 5. *G. juniperi-virginianae.* FIG. 6. *G. clavipes.* FIG. 7. *G. globosum.* FIG. 8. *G. nidus-avis.* FIG. 9. *G. bermudianum.* FIG. 10. *G. corniculans.* FIG. 11. *G. externum.* FIG. 12. *G. trachysorum.*

G. corniculans Kern, *G. externum* Arth. & Kern, and *G. trachysorum* Kern (Figs. 10, 11, and 12) are examples of species that are distributed in a narrow zone extending east and west across the eastern region. *G. corniculans* is found only in the northern extreme of the coincident ranges of its alternate hosts. *G. externum* has been reported from a narrow corridor that runs through the middle portion of the coincident range of its alternate hosts. *G. trachysorum* is a little known species reported from a few stations near the Atlantic Coast and the Gulf of Mexico in the United States.

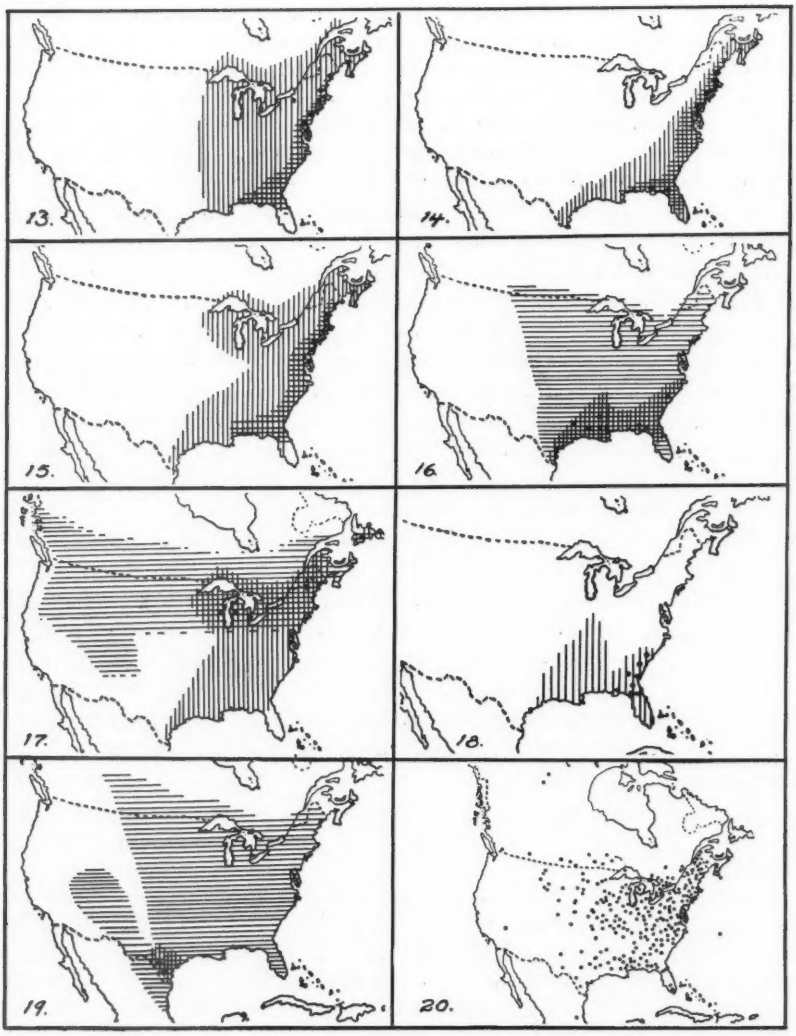
In contrast to the east and west distribution of the three species just discussed is the north and south distribution of three species whose telia occur on *Chamaecyparis thyoides*, namely, *G. biseptatum* Ellis, *G. ellisii* (Berk.) Farl., and *G. transformans* (Ellis) Kern (Figs. 13, 14, and 15). With the exception of the single inland station in the vicinity of Lake Ontario in New York, all other stations for these species are along the Atlantic Coast. Each of these species is most abundant in the northern part of its host's ranges, and it would seem that they are host-limited in this region. Between the Delaware peninsula and the Gulf of Mexico is an extensive area in which both kinds of alternate hosts are reported. *G. transformans* has not been reported from this area, but *G. ellisii* and *G. biseptatum* are found in the most southern portion of it, approximately one thousand miles south of the nearest northern station.

Three species of *Gymnosporangium* are definitely limited by the ranges of their aecial hosts. These are *G. floriforme* Thax. of the southern portion, *G. davisii* Kern of the northern portion, and *G. exiguum* Kern, intermediate between the eastern and western regions (Figs. 16, 17, and 19). Though fully as conspicuous as most other species, particularly on their aecial hosts, the few collections of these species indicate that they are among the rarest in eastern North America.

In eastern North America one species only, namely, *G. hyalinum* (Fig. 18), is as yet unconnected with a telial host. Its aecial hosts are species of *Crataegus*.

Two exotic species have been found in eastern North America. These are *G. haraeanum* Syd. and *G. japonicum* Syd. (Figs. 37 and 38), both native to eastern Asia. They were found on imported ornamental junipers most of which were destroyed shortly after examination at the port of entry. As the species have not been reported since, it is assumed that they did not become established. They did, however, become established on the Pacific Coast of western North America.

To complete the discussion of species of *Gymnosporangium* in eastern North America, it should be recalled that two of the tricontinental species, namely, *G. aurantiacum* and *G. clavariaeforme*, have been recorded from the region. Stations for species of *Gymnosporangium* of the eastern region have been plotted together in Fig. 20.



FIGS. 13 – 20. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for the rust.* FIG. 13. *G. biseptatum.* FIG. 14. *G. ellisii.* FIG. 15. *G. transformans.* FIG. 16. *G. floriforme.* FIG. 17. *G. davisii.* FIG. 18. *G. hyalinum.* FIG. 19. *G. exiguum.* FIG. 20. *Gymnosporangia of the eastern North American region.*

Species of the Western North American Region

A. Endemic species—

1. *G. betheli*
2. *G. cupressi*
3. *G. guatemalianum*
4. *G. harknessianum*
5. *G. inconspicuum*
6. *G. juvenescens*
7. *G. kernianum*
8. *G. libocedri*
9. *G. meridissimum*
10. *G. multiporum*
11. *G. nelsoni*
12. *G. nootkatense*
13. *G. speciosum*
14. *G. tubulatum*
15. *G. vauqueliniae*

B. Tricontinental species—

16. *G. aurantiacum*
17. *G. clavariaeforme*
18. *G. juniperinum*

C. Exotic species—

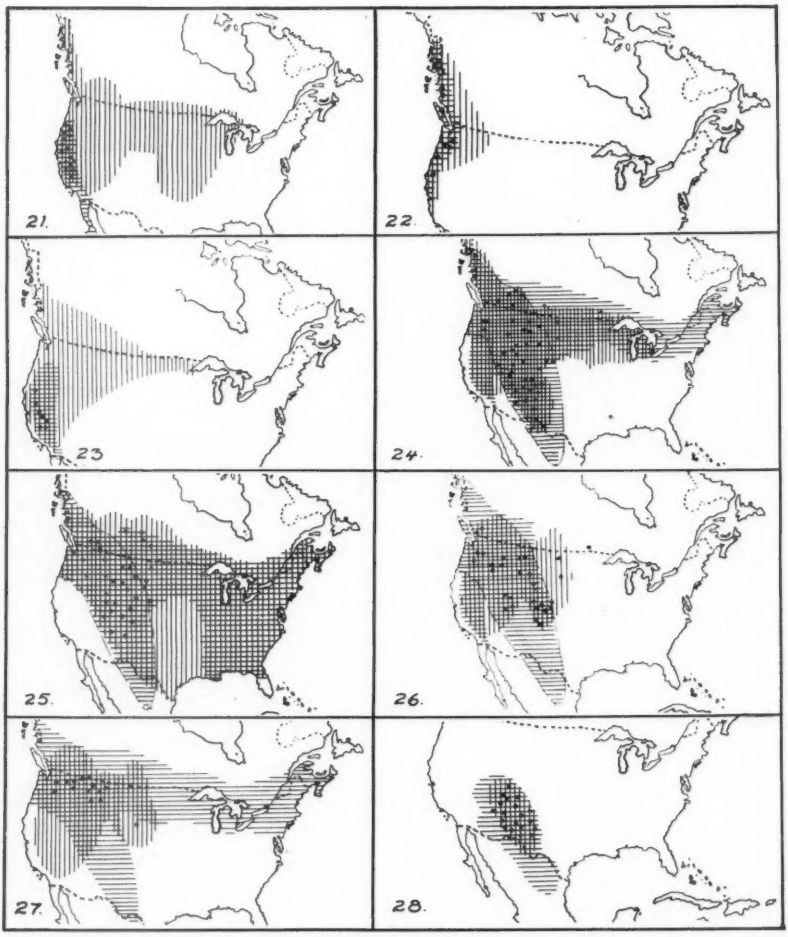
19. *G. haraeanum*
20. *G. japonicum*

This region vies with the eastern region in the richness and diversification of its *Gymnosporangium* flora. Twenty species are reported from the western region. These are made up of 15 endemic species, 2 exotic species (the same 2 from Asia that were found in the eastern region), and the 3 indigenous tricontinental species. Geographically the species are distributed in two distinct sub-regions corresponding to the two major mountain chains. Thirteen of the species (10 endemic and 3 tricontinental) are found in the Rocky Mountains while 5 (3 endemic and 2 exotic) are found in the Sierra Nevada and Cascade Mountains, and 2 species have recently been found in Guatemala. No species of *Gymnosporangium* is generally distributed through the whole of the western region, but the more widely distributed species occur in both mountain ranges, where the latter merge in the northern United States and southern Canada. It is interesting to observe that several species are found over the maximum available territory covered by the coincident ranges of their alternate hosts—a phenomenon not found elsewhere for this genus of rusts.

The species of *Gymnosporangium* in western North America will now be discussed in detail—first, those that occur in the Cascade and Sierra Nevada Ranges. *G. libocedri* (P. Henn.) Kern (Fig. 21) is one of the species that occupies all potential territory covered by the coincident ranges of its alternate hosts. Although few stations are recorded for *G. nootkatense* (Trel.) Arth. (Fig. 22), it also probably occupies all potential territory. *G. harknessianum* (E. & E.) Kern (Fig. 23) extends over a small portion of its potential territory.

Two exotic Asiatic species, *G. haraeanum* and *G. japonicum* (Figs. 37 and 38) seem to have become established along the Pacific coast parasitizing, however, exotic hosts only.

In the larger sub-region of western North America, the Rocky Mountain region, three species of *Gymnosporangium* are rather widely distributed from southern Alaska to New Mexico. *G. nelsoni* Arth. (Fig. 24) is the most widely distributed and abundantly represented species in the western region. Its alternate hosts, however, extend far beyond the range of the rust. *G. juvenescens* Kern (Fig. 25) is also generally distributed in the Rocky Mountains.



FIGS. 21 – 28. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for the rust.* FIG. 21. *G. libocedri.* FIG. 22. *G. nootkatense.* FIG. 23. *G. harknessianum.* FIG. 24. *G. nelsoni.* FIG. 25. *G. juvenescens.* FIG. 26. *G. betheli.* FIG. 27. *G. tubulatum.* FIG. 28. *G. speciosum.*

Of rather scattered distribution is *G. betheli* Kern (Fig. 26). Its stations tend to be clustered together in small groups, indicative of localized centres for the rust. This may be explained in part by the fact that certain of its aecial hosts—species of *Crataegus*—are said to prevail chiefly near lakes and streams.

All other endemic species of *Gymnosporangium* in the western region are of local distribution. One of these, *G. tubulatum* Kern (Fig. 27) is confined

to the more northerly portion of the coincident ranges of its alternate hosts. Three species, *G. speciosum* Peck, *G. kernianum* Bethel, and *G. inconspicuum* Kern (Figs. 28, 29, and 30) are found over all potential territory. *G. speciosum* is one of the most abundantly represented species in the southern portion of the Rocky Mountains. It doubtless extends southward into Mexico, yet no station is recorded for it there. *G. kernianum* is found over essentially the same area as *G. speciosum*, though it appears to be less common. *G. inconspicuum*, like the two species just discussed, is limited to the southern portion of the Rocky Mountains. It occupies all potential territory.

Four species in western North America are as yet unconnected with alternate hosts. *G. cupressi* Long and Goodding (Fig. 31) is probably of limited distribution, since its telial host *Cupressus arizonica* Greene is confined to southwestern Arizona. *G. meridissimum* Crowell and *G. guatemalianum* Crowell (also Fig. 31) are known from a few stations in Guatemala. Since no other species of *Gymnosporangium* is known from Guatemala, these may represent a single species. *G. multiporum* Kern (Fig. 32) though probably limited to the southern Rocky Mountains may be of wider distribution. *G. vauqueliniae* Long & Goodding (also Fig. 31) is known from the type locality only in southern Arizona. It will be recalled that the tricontinental species are scattered through the Rocky Mountains, and in the northern portions are found generally over the western region. Stations for endemic species of *Gymnosporangium* in western North America, except the Guatemalian ones, are shown in Fig. 33.

Species of the Asiatic Region

A. Endemic species—

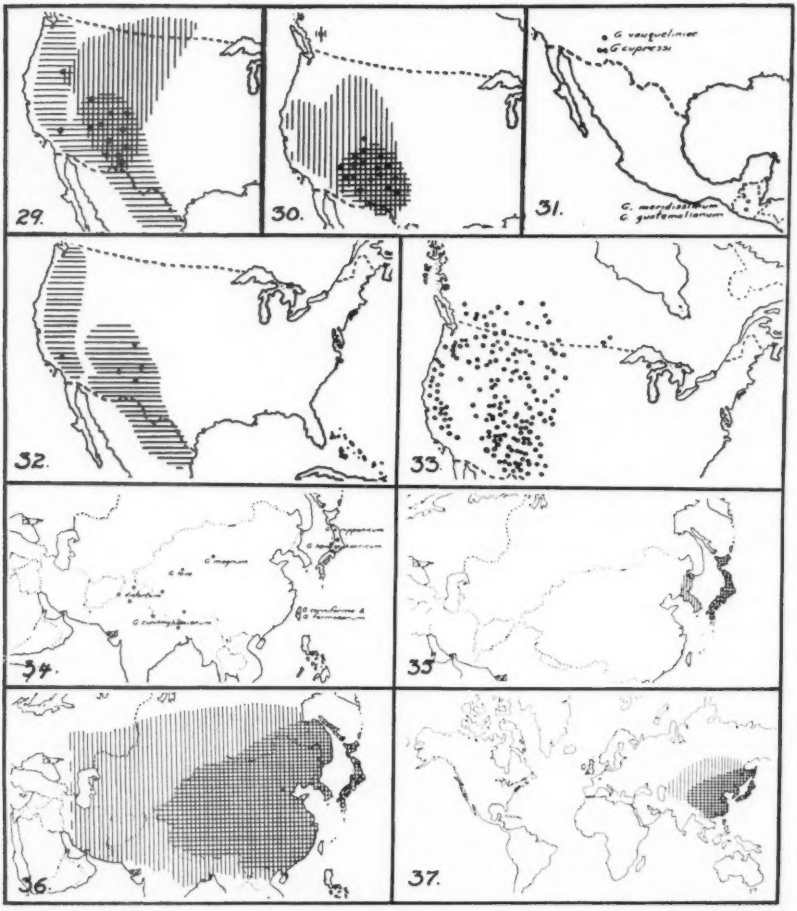
1. *G. corniforme*
2. *G. cunninghamianum*
3. *G. distortum*
4. *G. formosanum*
5. *G. haraeanum*
6. *G. hemisphaericum*
7. *G. japonicum*
8. *G. leve*
9. *G. magnum*
10. *G. miyabei*
11. *G. nipponicum*
12. *G. yamadae*

B. Tricontinental species—

13. *G. aurantiacum*
14. *G. clavariaeforme*
15. *G. juniperinum*

The mycological flora of Asia is probably less known than that of other geographical regions, yet the *Gymnosporangium* flora, though sparsely represented in number of collections, is so abundant in number of species in widely separated stations as to indicate a richness that may rival that of North America.

Fifteen species of *Gymnosporangium* are reported from Asia. Twelve are endemic and three are indigenous. Eight of the endemic species are known from the type locality only or from a few stations in the vicinity. These species are *G. cunninghamianum* Barcl. in northeastern India and southern Tibet (6 stations recorded, 3 only could be plotted); *G. distortum* Arth. & Cummins from northern India and western China (4 stations, 3 plotted); *G. leve* Crowell and *G. magnum* Crowell known from their type localities in



FIGS. 29 – 37. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for rust.* FIG. 29. *G. kernianum.* FIG. 30. *G. inconspicuum.* FIG. 31. *G. cupressi. G. guatemalianum, G. vauqueliniae, G. meridissimum.* FIG. 32. *G. multiporum.* FIG. 33. *Gymnosporangia of the western North American region. (Guatemalian stations not shown, see Fig. 31.)* FIG. 34. *Shows stations for 8 species of Gymnosporangium in Asia.* FIG. 35. *G. miyabei.* FIG. 36. *G. yamadae.* FIG. 37. *G. haraeanum.*

China; *G. nipponicum* Yamada (type locality); *G. hemisphaericum* Hara (2 stations, both plotted) of Honshu, Japan, and *G. corniforme* Sawada and *G. formosanum* Hirat. known from their type localities in Formosa. Stations for these eight species have been plotted on Fig. 34.

Two endemic species are of wide distribution in Japan, *G. miyabei* Yamada & Miyake and *G. yamadae* Miyabe (Figs. 35 and 36).

Two species, seemingly endemic to Asia, have been imported to both North America and Europe; these are *G. haraeanum* Syd. and *G. japonicum* Syd. (Figs. 37 and 38). Both species are found in Japan and coastal China. Of much interest was the discovery of these species on junipers imported in Connecticut about 1910. The fungi have not been reported from eastern North America since. In western North America, these rusts have become established on Asiatic hosts and were probably imported on one or both alternate hosts. Recently these species have been reported from France; *G. haraeanum* was reported on *J. chinensis* and *G. japonicum* on *Pyrus sinensis* Lindl.

The three indigenous tricontinental species have been collected at several stations in Japan. Each of the species attacks endemic and indigenous hosts; this would seem to indicate that they occur naturally in Asia rather than being introduced. Stations for the 12 endemic Asiatic species are plotted in Fig. 39. The concentration of stations in Japan and the west coast of Asia is probably due to the more intensive mycological studies carried on there.

Species of the European Region

A. Endemic Species—

1. *G. confusum* 2. *G. minus* 3. *G. sabinae*

B. Tricontinental Species—

4. *G. aurantiacum* 5. *G. clavariaeforme* 6. *G. juniperinum*

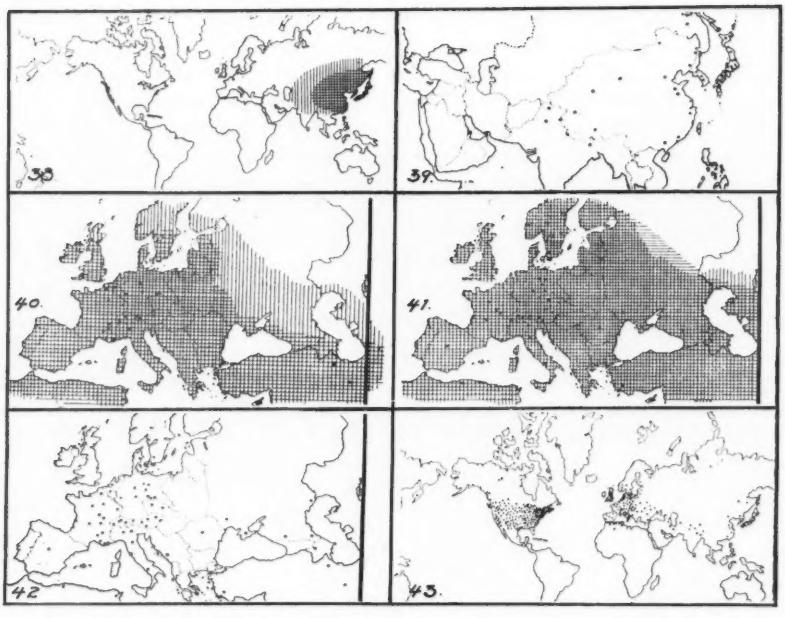
C. Exotic Species—

7. *G. globosum* 8. *G. haraeanum* 9. *G. japonicum*

This region, including north Africa, though the smallest of the geographical regions, contains a disproportionally meager *Gymnosporangium* flora. Nine species occur in Europe. Of these, three have been introduced, but are probably not established—*G. globosum*, *G. japonicum*, and *G. haraeanum*. Each of the three tricontinental species, *G. aurantiacum*, *G. clavariaeforme*, and *G. juniperinum* are generally distributed over much of the European region. The recently described species *G. minus* Crowell on *Cupressus sempervirens* L. is known from the type locality in Athens, Greece.

Thus, of the nine species reported from Europe two only, *G. confusum* Plowr. and *G. sabinae* (Dicks.) Wint. [*G. fuscum* Hedw. F., *G. tauricum* J. Ericks.] (Figs. 40 and 41) are endemic and generally distributed in Europe. Each is doubtless present in greater abundance than represented on the maps. Scores of records are at hand that cannot be plotted because of insufficient information.

In Fig. 42, stations for the three endemic European species, *G. confusum*, *G. sabinae*, and *G. minus* are plotted. An examination of this map will show that they are distributed throughout much of central Europe. The scattering of stations eastward to the Black and Caspian Seas seems to indicate a still farther extension into Asia.



FIGS. 38 – 43. *The geographical distribution of Gymnosporangium in relation to that of alternate host groups. Vertical lines, aecial hosts; horizontal lines, telial hosts; dots, stations for rust.* FIG. 38. *G. japonicum.* FIG. 39. *Gymnosporangia of the Asiatic region.* FIG. 40. *G. confusum.* FIG. 41. *G. sabinae.* FIG. 42. *Gymnosporangia of the European region.* FIG. 43. *Gymnosporangia of the Northern Hemisphere.*

Explanation of the Types of Geographical Ranges of Species of Gymnosporangium

The *Gymnosporangium* floras of Asia and Europe are of little value for the purposes at hand, since in the former region the flora is scantily represented and in the latter the few widely distributed and one localized species offers little variation for discussion. The *Gymnosporangium* flora of North America composed of several species of wide geographical ranges affords excellent opportunities for observation and comparisons. The types of geographical ranges for species of *Gymnosporangium* may be classified in four categories as follows:—

(A) Species that occupy all potential territory covered by the coincident ranges of their alternate hosts.

(B) Species that occupy less than all potential territory.

1. Species that are confined by the range of their "primary" telial host.
2. Localized species that are confined within a portion of the coincident ranges of their alternate hosts.

3. Widely distributed species that are not limited in their range by either alternate host group.

These categories will be discussed in the order of their arrangement in the key.

(A) Species that Occupy All Potential Territory Covered by the Coincident Ranges of Their Alternate Hosts

Species in Category *(A)* are found in Western North America only and include *G. libocedri*, *G. nootkatense*, *G. inconspicuum*, *G. kernianum*, and *G. speciosum* (Figs. 21, 22, 28, 29, and 30). These species are definitely and sharply limited by the coinciding ranges of their alternate hosts. With the exception of the eu-form species *G. nootkatense*, they have no means of perpetuating themselves beyond the coinciding ranges of their alternate hosts. *G. nootkatense* occurs over the full extent of its II-III host and hence occupies all potential territory.

(B) Species that Occupy Less than All Potential Territory

1. Species that are confined by the range of their "primary" telial host.

The phrase "primary telial host" requires explanation. The writer's unpublished study of hosts of the genus *Gymnosporangium* has revealed that one species of *Juniperus* in each geographical region had all or most of the following characteristics: *(a)* it appeared to be the most susceptible host, *(b)* it was attacked by most or all the species of *Gymnosporangium* parasitizing hosts in the section Sabina of the genus *Juniperus*; *(c)* it was the most widely distributed species. In eastern North America, *Juniperus virginiana* was the most susceptible telial host; it was attacked by all the species of *Gymnosporangium* parasitizing hosts in the section Sabina; it occurred over the greater portion of the eastern region. These statements are largely true of *J. scopulorum* in western North America. *J. chinensis* appears to be the primary telial host in Asia. A primary telial host can hardly be said to exist for the few species of *Gymnosporangium* in Europe. Other telial hosts may be called "supplementary" telial hosts, and are characterized by being more resistant, by being attacked by fewer species of *Gymnosporangium*, and by being of more local distribution. Not all species of these rusts have supplementary telial hosts, for many are known to occur on the primary telial hosts only.

Six species are placed in Category (1)—*G. juniperi-virginianae*, *G. globosum*, and probably *G. nidus-avis* of eastern North America; *G. nelsoni*, *G. juvenescens*, and *G. betheli* of western North America (Figs 5, 7, 8, 24, 25, and 26). In order that a rust maintain itself in numerical equilibrium over a period of years, one infection must produce another. An essential requirement in this genus appears to be the high susceptibility of the primary telial host. An illustration of this phenomenon may be taken from the reaction of *Juniperus* sp. to infection by *G. juniperi-virginianae*. In nurseries in Massachusetts where various species of junipers grow in large numbers close together it was repeat-

edly observed that the primary telial host, *Juniperus virginiana*, invariably supported the preponderance of infections of *G. juniperi-virginianae*. While other junipers would be attacked, one often had to search for the isolated infections. Such resistance of supplementary telial hosts appears to prevent the rust from perpetuating itself over territory occupied by them, but in which the primary telial host is not found. An examination of the maps of distribution of the species in Category (1) will show that the ranges of the rusts coincide closely with the ranges of the primary telial hosts—which are outlined on the maps by means of a broken line. A minor exception should be noted for *G. betheli* (Fig. 26), whose aecial hosts do not extend into the southern portion of the range of the primary telial host, though the rust extends as far south as the aecial hosts permit.

According to the theory it is unlikely that *G. juniperi-virginianae*, the cedar-apple rust fungus, will extend into such apple growing regions as the Maritime Provinces or the Pacific States where, although alternate hosts are present in abundance, the primary telial host is absent. The theory also offers an explanation as to how *G. japonicum* and *G. haraeanum* of Asia became established on the Pacific Coast of North America—their primary telial host, *J. chinensis*, (and aecial hosts) were imported and became established earlier than the rusts or at the same time.

2. Localized species that are confined within a portion of the coincident ranges of their alternate hosts.

In Category (2) are placed seven exemplary species—*G. bermudianum*, *G. corniculans*, *G. externum*, *G. trachysorum*, *G. floriforme*, *G. davisii*, and *G. tubulatum* (Figs. 9, 10, 11, 12, 16, 17, and 27). It will be observed that each of these species has its maximum range in an east and west direction. This is also the direction of isothermic lines of spring temperatures in the region of distribution of these rusts. Hence temperature is probably a potent factor influencing the range of these species. That temperature alone probably does not restrict the geographical ranges of these species of *Gymnosporangium* is indicated by the finding that the temperature range for sporulation of most species is from 3° to 30° C.—a range wider than that which usually occurs during the spring weeks, when teliospores germinate, and the summer and fall months, when aeciospores probably germinate. That humidity alone probably does not restrict the geographical ranges of these species of *Gymnosporangium* is indicated by the fact that rains regularly occur during the critical sporulating periods. As they are remarkably resistant spores, the time over which favourable temperatures and humidity conditions can occur can be of several weeks duration. It has been observed by several authors that teliospores may be mature and a high percentage of them germinate well in advance of production of new, susceptible growth by aecial hosts. However, as most species of *Gymnosporangium* are perennial on their telial hosts, should favourable conditions fail to occur in one year, the rust can abide the fortunes of one and probably more subsequent years.

It has been shown that aecial hosts of *G. globosum*, *G. juniperi-virginianae*, and *G. clavipes* (4, 5, 12) have a definite period during which they can become infected. This period of susceptibility usually extends from a few days to a few weeks after growth begins in the spring. That a period of susceptibility exists for hosts of any species in Category (2) is not definitely known, but one is presumed to exist for each aecial host. It has been observed that the period of susceptibility of some aecial hosts is passed during the spring, when humidity conditions did not permit infection to occur. These phenomena of temperature, spore germination, precipitation, and period of susceptibility of aecial hosts in the spring materially lessen the possibilities of infection by species of *Gymnosporangium*. Based on these phenomena, it is suggested that the narrow geographical range of species of *Gymnosporangium* in Category (2) represents the zone over which favourable environmental conditions consistently occur during the period of susceptibility of the aecial hosts as the spring season advances northward.

Four additional species of *Gymnosporangium*—*G. biseptatum*, *G. ellisii* and *G. transformans* of eastern North America and *G. harknessianum* of the western region (Figs. 13, 14, 15, and 23) probably belong in this category or are a distinct group. Their distribution differs from that of the others in that the single telial host of each species seems to be the limiting host but not the limiting factor in their distribution. The first three species are definitely telial-host limited in the northern portion of their range. Very disturbing are the stations for *G. biseptatum* and *G. ellisii* in the southern portion of eastern North America, approximately 1000 miles away from the nearest station for the rust. It seems unwise, with the meagre data available, to hazard an explanation of the geographical distribution of these four species.

Several species of *Gymnosporangium* as *G. nelsoni*, *G. juvenescens*, *G. betheli*, *G. kernianum*, and *G. inconspicuum* (Figs. 24, 25, 26, 29, and 30) are reported to be found at stations far removed from the main centres. Some of the collections have been carefully checked. How they occurred does not seem to be clear, for none of these species can maintain itself on one host. Could they be due to the chance finding of sporadic infections resulting from wind, bird, or insect carried spores? It would be interesting, indeed, to have accurate data on the duration and extent of the infections in such isolated places. Some of the isolated stations for certain species are subject to question, for it is difficult, if not impossible, to identify certain species of *Gymnosporangium* from the aecial or telial phase alone.

3. Widely distributed species that are not limited in their range by either alternate host group.

Some species of *Gymnosporangium* in Category (3), namely, *G. aurantiacum*, *G. clavariaeforme*, and *G. juniperinum* (Figs. 1, 2, and 3) seem to owe their wide distribution in large measure to the facts that the telial host, *J. communis*, is found throughout the temperate portion of the northern hemisphere, and that these species parasitize from 10 to 51 species of aecial hosts in 3 to

12 genera, some of which are found in association with the telial host in all the northern continents. However, host associations alone do not seem sufficient to explain the peculiarly wide distribution of these species. The irregular zones of distribution shown on the maps would seem to indicate that temperature, humidity, and geographical factors are of much influence.

Gymnosporangium clavipes (Fig. 6) probably belongs in this category, but is limited in its geographical range to North America. While this is primarily an eastern North American species it has several stations in western North America and is not limited in its range by either alternate host group. Its alternate hosts, including *J. communis*, *J. virginiana*, and many of its more than 400 aecial-host species, occur in association throughout the greater portion of the northern hemisphere. It appears that this species has potentialities for increasing its geographical range. Since new stations are appearing in western North America from time to time, it would appear that it is actively doing so. This phenomenon has not been observed with any other species of the genus.

Discussion

Bisby (3) concludes from his analyses of geographical distribution of fungi that, in general, fungi have a wider distribution than flowering plants. The investigation on geographical distribution of the genus *Gymnosporangium* would seem to support conclusions quite the reverse of Dr. Bisby's general finding, as indicated by the three following observations. (i) Few species of *Gymnosporangium* occupy all potential territory covered by the coinciding ranges of their alternate hosts, which in each instance is of limited extent (Figs. 21, 22, 28, 29, and 30). (ii) Although the tricontinental species are of wide distribution, they fall far short of occupying all potential territory, They seem to be most abundant in Europe and of local occurrence in North America and Asia (Figs. 1, 2, and 3). (iii) Most species of this genus are of local distribution. They are not limited by the coinciding ranges of their alternate hosts and few species are limited geographically by one or the other of their hosts. With two well established exceptions only, *G. haraeanum* and *G. japonicum*, species are confined to a single continent.

Overholts (13, p. 631) is of the opinion that temperature and moisture must be among the predominant factors influencing the geographical distribution of certain polypores. There seems little doubt but that Overholts' evaluation of factors influencing the geographical distribution of polypores in North America is also largely applicable to the genus *Gymnosporangium*. But there seem to be additional limiting factors of much importance to this genus. All species of this genus are obligate parasites and all but two lack the repeating uredial stage; hence they can survive only within the area occupied by the coinciding ranges of their alternate hosts.

Temperature acting upon the parasite does not seem to be of paramount importance as a limiting factor. This is *indicated* by the finding that spores of many species will germinate over a greater range than that in which the

species live. Humidity acting upon the parasite also does not seem to be of paramount importance as a limiting factor. If humidity conditions are not favourable, even over the whole range of most species for one or a few years, the perennial nature of the parasite in the telial host will offset such an abnormal occurrence. A brief period of susceptibility during which aecial hosts can become infected has been shown to exist for certain species of *Gymnosporangium*, and one is assumed to exist for all aecial hosts of this genus of rusts. This factor is probably a potent one in regulating geographical range. If a species has a large number of aecial hosts with varying durations of the period of susceptibility and varying lateness for breaking bud, the time over which infection can occur is thereby lengthened and the potential geographical range extended. The reverse situation would also seem to be true.

It has been observed that the maturation of teliospores in the spring does not coincide with exactness with the production of new susceptible growth by aecial hosts. The writer's observations show that teliospores of *G. clavipes* may be germinable one to four weeks prior to the breaking of buds of aecial hosts under conditions in Massachusetts. In 1932, showers were so frequent, after teliospores of *G. clavipes* were germinable, that the majority of teliospores had germinated before buds of aecial hosts had broken; hence few were left to cause infection. So few, in fact, that *Aronia melanocarpa*, a susceptible shrub that produced foliage and flowers rather late in the spring, was not infected that year in the several areas visited, where plants were infected the year previous and in later years. The phenomenon of maturation and germination of teliospores prior to production of susceptible growth by aecial hosts has been observed with several species of *Gymnosporangium* in Massachusetts and New Mexico.

A species could not survive in any area where teliospores would regularly mature much later than buds break on aecial hosts, for the brevity of the period of susceptibility would render the new growth non-susceptible.

Geographical barriers are potent obstacles to the geographical ranges of most species of *Gymnosporangium*. These barriers, however, seem to have their effect by restricting the range of hosts, principally of the primary telial host. Primary telial hosts are endemic to each of the geographical areas discussed in the body of this paper, and certain species that are largely confined to one of these hosts are also restricted to its range. *Juniperus communis* is the only telial host to occur circumpolarly. It is interesting to observe that three rusts on this host are also circumpolar. However, *G. davisii*, which parasitizes *J. communis*, is of local distribution, while *G. clavipes*, which consistently parasitizes *J. communis* (and *J. virginiana*), is widely distributed in North America, though principally in the eastern region.

From the writer's observations to date it appears that no one factor is paramount in importance in governing the range of most species of *Gymnosporangium*. It appears vital that several phenomena, perhaps especially suitable temperature and humidity conditions and the existence of germinable

teliospores, must coincide with annual regularity during the period of susceptibility of aecial hosts in order that a species maintain itself within a geographical area where alternate hosts are associated.

Acknowledgments

The author acknowledges with pleasure the aid extended by Dr. M. L. Fernald, Gray Herbarium, Harvard University, in the early stages of this study; by Dr. J. H. Faull, the Arnold Arboretum, Harvard University; and by Prof. J. G. Coulson, Macdonald College, McGill University, for suggestions and criticism of the manuscript.

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PARTIAL CONGENITAL OCCLUSION OF THE VAGINA IN THE SILVER FOX[1]

BY BENJAMIN KROPP[2]

Abstract

A condition of partially imperforate vagina in the silver fox is described and briefly discussed.

The present report deals with a congenitally anomalous urogenital orifice encountered in the silver fox (*Vulpes fulva fulva*). Two different males had been placed successively with the mature vixen, and each male had failed to impregnate her, owing apparently to failure of the female to receive the male intromittent organ. The owner then sacrificed the animal for its pelt since it was worthless as a breeder, and the carcass, in a frozen state, was turned over to the writer for examination.

The opening into the urogenital canal was found to be covered transversely by a dome-shaped septum that completely covered the external aperture except for a small opening at the extreme left lateral margin (Fig. 1). The

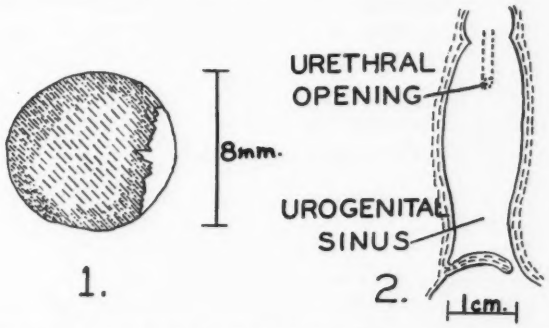

FIG. 1. *Diagrammatic external view of membrane covering urogenital aperture.* FIG. 2. *Diagrammatic frontal section of urogenital sinus and membrane. See text for explanation.*

septal edge bordering the marginal opening was uneven and serrated. The total diameter of the outer aperture in a lateral plane was 0.9 cm., and 0.8 cm. in an antero-posterior plane. The jagged free left edge of the septum

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reached to within 1.5 mm. of the left lateral margin. The membrane was tough and its external surface appeared cornified, although the membrane yielded readily to cutting with scissors. The concavity of the membrane may have been due in part to pressure of the penis during repeated attempts at copulation, but is to be attributed mainly to the effects of post-mortem shrinkage.

Unfortunately, the carcass was not fresh when received so that attempts to determine the nature of the occluding septum microscopically were not undertaken. However, the vagina and associated structures were dissected grossly and the septum found to be separable into two membranous sheets (Fig. 2), the inner sheet continuous with the vaginal lining, the outer sheet continuous with the skin surrounding the opening. Between the two sheets was a very thin layer of loose fibrous material, devoid of blood vessels, which sent small strands into the vaginal musculature. The ovaries, both horns of the uterus, and the fallopian tubes were grossly normal; no corpora lutea were detected in the ovaries. The urinary bladder opened into the urogenital sinus 28 mm. above the membrane, and the opening present in the latter was apparently sufficient to permit escape of urine. It is interesting to note that the presence of the septum could easily have been detected in the living animal if occlusion of the genital tract had been suspected as a possible cause of failure to mate. Removal of the structure with fine scissors could have been effected easily and sacrifice of the animal avoided.

Complete congenital occlusion of the vagina has been frequently encountered in laboratory colonies of adult rats (2) and mice (1). While in these and in other instances the congenital anomaly may vary in form, there is some reason to believe that in rats and mice the condition has an hereditary basis. The condition here described does not seem to be strictly comparable with that in rats and mice. The anomaly is regarded only as a developmental arrest in that the delamination of the vaginal plate failed to continue to completion.

Acknowledgment

The author is indebted for the specimen to Dr. N. V. Freeman.

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EXPERIMENTAL STUDIES ON *STRONGYLOIDES AGOUTII* IN THE GUINEA PIG[1]

By Henry J. Griffiths[2]

Abstract

The suitability and tolerance of the guinea pig to infection with *Strongyloides agoutii* presented an opportunity for the study of the bionomics of this species in an experimental host.

Serial transfer of this nematode through the guinea pig yielded a mixed type (free males and filariform larvae) of free-living development in faecal cultures which occasionally reverted to the indirect mode common to *S. agoutii*. A reversion to the indirect mode of development was produced when ova from faeces of guinea pigs infected with *S. agoutii* were cultured in sterile agouti faeces.

The termination of the prepatent period of *S. agoutii* in the guinea pig was shown to range from 7 to 10 days, and 71% of 58 animals were positive by faecal test by the eighth day. The patent period ranged from three to eight weeks.

The guinea pig was shown to develop an absolute acquired immunity to re-infection with *S. agoutii*. This resistance has been retained over a period of at least 6 to 13 months. An age resistance was not observed in animals one year old and over.

Introduction

Previous studies (10) on the morphology and biology of *Strongyloides agoutii*, of the golden-rumped agouti *(Dasyprocta agouti)*, led to further investigation on the experimental infection of some common laboratory animals with this species.

For some time it has been known that the infection of certain abnormal hosts with *Strongyloides* may produce radical changes in the mode of free-living development. Brumpt (3) observed an alternation of the free-living cycle when he infected rabbits with *S. papillosus* of sheep. In sheep he observed a great predominance of females in the bisexual generation; in fact, free males were a comparative rarity. However, in rabbits the males were produced in large numbers, even exceeding the females, whereas the production of filariform larvae was strikingly reduced. Similar results were observed by the writer in cross-infection experiments on rabbits with the sheep strain of *Strongyloides*.

Sandground (13) recorded peculiar results through establishing *S. fülleborni* and *S. stercoralis* in abnormal hosts; the former, which normally showed only the pure indirect type of development, on inoculation into dogs and man produced predominantly the direct type. Observations of a similar nature were recorded by Faust and Kagy (6), who used human and non-human primate strains of *Strongyloides*. They transferrred these strains to human volunteers, a macaque monkey, and a number of dogs, and their

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Assistant.*

results further demonstrated the instability of the free-living phase in hosts other than normal.

Accordingly, infection with *S. agoutii* was attempted in the guinea pig, which was selected for several reasons. Firstly, the guinea pig is a member of the same family as the agouti, and for that reason it was hoped that the presence of *S. agoutii* would be tolerated by this host. Secondly, the guinea pig does not appear to be readily infected with most helminth parasites and had been but little used as an experimental animal in helminthological research. As far as the writer is aware, there are only three recent references to infection of the guinea pig with *Strongyloides:* Krediet records an undetermined species from it; Brumpt (3) transferred *S. papillosus* from sheep to guinea pigs; and Sheldon and Otto (17) infected them with *S. ratti*, while testing the host specificity of this parasite.

Materials and Methods

The source of supply of *Strongyloides* material for this work was the golden-rumped agouti (*Dasyprocta agouti*), a rodent native to Trinidad, B.W.I., and northern South America. The housing, feeding, and handling of these rodents were described in a previous paper (10), as were the methods of making and examining the routine faecal cultures.

As a general rule, experimental animals were infected percutaneously with filariform larvae of *S. agoutii*, usually obtained from the lid of a Petri dish to which they migrated readily from the faecal mass. If not so readily available, the Baermann isolation technique was adopted with modifications. The majority of the larvae were isolated within an hour, provided the culture was not unduly thick and massive; by careful disposition of the culture, larvae and other free-living forms may be drawn off comparatively free of debris. This procedure would not be applicable to large masses, but is well adapted to small faecal cultures containing large numbers of larvae. *Strongyloides* larvae become quickly inactivated if stored in deep water. Therefore, it is preferable to draw them off frequently into Syracuse watch glasses and concentrate immediately prior to use for infection purposes.

A modification of this technique was also adopted as a confirmatory measure in some post-mortem examinations. On certain occasions it was desired to determine whether larvae of immature adults were present in the lungs, heart, and portions of small intestine.

As a general rule an anaesthetic was given when larvae were administered percutaneously. For complete anaesthesia, Nembutal was injected intraperitoneally at the rate of 1 cc. per 5 lb. of live weight; anaesthesia was complete in a few minutes, and lasted from 1 to 3 hr. A greater measure of success was observed by percutaneous than by oral administration of larvae. The guinea pigs were approximately eight weeks old unless required older for some specific experiment. A small area of about one square inch was shaved on the animal's abdomen, rinsed with water to remove soap, then with alcohol,

and allowed to dry. The infective larvae were applied to the spot by means of a pipette, and the area was supplied with larvae and moisture for 15 to 20 min. It was then permitted to dry and the guinea pig kept under observation until again active. On no occasion did this method fail to establish a positive infection when suitable host animals were employed.

The eggs of *S. agoutii* are in the late stages of segmentation or embryonated when evacuated in the faeces. The routine faecal examination was used for diagnostic purposes only; quantitative output of ova was not considered necessary for the interpretation of results. A sugar centrifugal-flotation technique was used, and about 1 cc. of faeces was taken for examination.

For collection of faeces the animals were kept individually in wooden cages with $\frac{1}{4}$ in. wire mesh bottoms. The faeces dropped through onto paper or into water, as required. At times it was desirable to collect the total output for several days. A four-sided funnel of sheet zinc was attached to the bottom of the cage by hooks; the seams of the zinc were soldered so that daily washing would remove dirt or urine sediment. A beaker was placed beneath to collect the pellets.

All infected animals were kept in metal cages with wire floors, and no contamination or re-infection of an individual was experienced throughout these studies. The guinea pigs were identified by their colour markings, as only small numbers were used at a time. Tattoo marks were used in the ears of rabbits and the ears and tails of rats and mice were cut for identification.

Prior to post-mortem examination, the animals were starved for at least 12 hr. The entire small intestine was removed by cutting at the pyloric end of the stomach and at the caecum. The duodenum was then separated and the remaining length of intestine divided into two approximately equal parts. Since the number of individuals obtained at autopsy was not significant in any of these experiments, a method of collecting and fixing the adults en masse was adopted. The portion of intestine was slit open with a sharp scissor blade and covered with normal saline in a small Erlenmeyer flask. After half an hour the flask was stoppered and shaken gently. This procedure was repeated two or three times and the intestine removed. Most of the worms were detached by this time and found among the floating mucus. The contents of the flask were poured slowly into a very fine tea strainer, which held back most of the nematodes, or into a strainer covered with bolting cloth, which retained all.

The worms were carefully washed off by a fine jet of water into a petri dish, in which they were preserved. The majority of the worms parasitized the upper half of the small intestine of the guinea pig, the greatest concentration being found in the duodenum and the first few inches of the jejunum. Adults were obtained frequently from the ileum but only on rare occasions from the caecum or colon.

All animals used (with the exception of the agouti) were born and raised at this Institute under conditions that precluded any possibility of previous infection with *Strongyloides* species.

The Infection of an Experimental Host with *S. agoutii*

The tolerance of the guinea pig to *S. agoutii* and its desirable qualities as a laboratory animal provided ample opportunity for investigation.

Experiments were outlined to yield data on the minimum time required for parasitic females to reach maturity, to note the type of free-living development that would occur after the passage of the strain through an experimental host, and to record the length of life of the infective larvae.

After primary infection with the agouti strain, infections were transferred serially from one guinea pig to the next. This procedure excluded the possible introduction of a new strain into the series and any change occurring in mode of development could thus be attributed to some change in type. Owing to the peculiar results recorded, it was considered desirable to repeat the serial infection of guinea pigs, and two analogous series were started.

By preliminary trial, the prepatent period of the parasitic females was found to be approximately eight days. Faeces samples were examined from the sixth day following infection. Faecal tests were continued daily until the individual showed a heavy output of ova, whereupon examinations were made weekly until the test was negative for two or three weeks, when examination was discontinued. The exact date that ova ceased to be passed was not recorded. On several occasions experimental animals appeared to become constipated at the time the female parasite commenced ova production. This unavoidable condition extended the prepatent period for one day or longer, in some instances.

As soon as large numbers of ova were detected, three samples per individual were collected on different days for culturing to observe the mode of development.

Because the ova are in the late stages of segmentation when expelled, it was not considered necessary to examine cultures for 20 to 24 hr. Examination of cultures was concluded as soon as the activity of the filariform larvae ceased. It will be noted that the total number of cultures recorded graphically does not always correspond exactly with the total number of cultures examined. These omissions were unavoidable on account of sickness and holidays which occurred during the investigation. Some animals employed in these studies died as a result of over-infection, some were killed to obtain specimens of the parasitic generation of *S. agoutii*, and others were held for future use in immunity studies.

Serial Infection of the Guinea Pig. Series I

In this series, serial transfer of the agouti strain of *Strongyloides* was made through 27 guinea pigs. The experiment extended from April 1936 to November 1937, and during that period 92 cultures were made and 1,314 observations recorded.

Filariform larvae used for infective purposes ranged from 4 to 17 days in age, though the majority were from 4 to 6 days old. All the animals were

young males except guinea pig No. 21, a three-months-old female. Cultures from No. 24 were not examined in detail, but filariform larvae were obtained for transfer purposes. No. 27 died suddenly, terminating this series.

Transfer of *S. agoutii* through the guinea pig resulted in a marked change in the mode of free-living development. In the agouti, this strain showed an indirect or heterogonic mode of development, whereas in the guinea pig a mixed type was observed in the first 19 guinea pigs. The term "mixed" is used to indicate that the development observed was not entirely direct, for in addition to the production of filariform larvae, free-living males made their appearance. Only occasionally were no males found.

This mode of development did not persist throughout the entire series, since cultures from No. 20 showed the presence of a few free females in addition to the filariform larvae and free males, a modified reversion to the indirect type of the agouti. Cultures from No. 21 continued to show the presence of free females in quite large numbers, though cultures from Nos. 22, 23, 25, and 26 produced only filariform larvae and free males.

This mixed type of development presented an excellent opportunity for recording the period of activity of free males, the first appearance of filariform larvae of direct development, and the longevity of these larvae, in faecal cultures. Such data are recorded in Figs. 1–4. Free males occurred in the majority of cultures on the second and third days, but only once on the first day; they did not appear after the fourth day (Fig. 1). In the greater number of cultures the males became inactive on the fourth and fifth days, though in one instance they were active on the seventh day. In only one instance were males observed in cultures for more than four days, the longevity in most cases being from two to three days. Filariform larvae occurred in the majority of cultures on the third day, though many were recorded on the second and fourth days; in a few instances they did not appear until the fifth day (Fig. 2). The duration of life of filariform larvae in cultures was easily recorded with this mixed type of development.

FIG. 1. *Frequency of first day of appearance and last day of activity of free-living males of S. agoutii in faecal cultures from the guinea pig.*

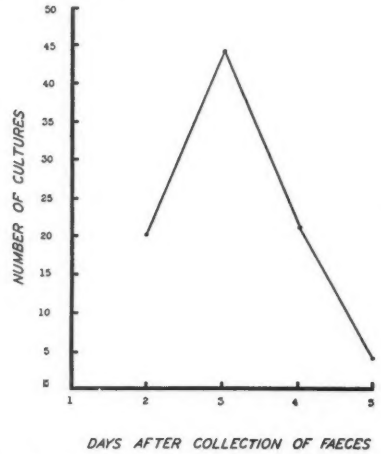

FIG. 2. *Appearance of filariform larvae of S. agoutii in cultures after passage of faeces by guinea pigs.*

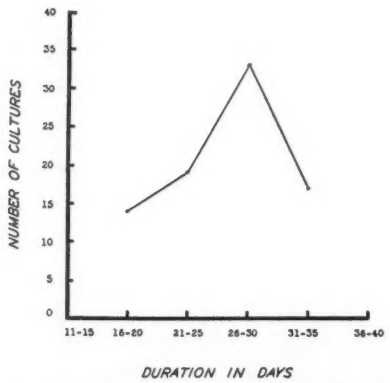

FIG. 3. *Longevity of filariform larvae of S. agoutii in faecal cultures from the guinea pig.*

Fig. 3 indicates that the greater proportion of cultures died between the 26th and 30th days, while no cultures became inactive prior to 16 days, or continued to show active larvae after 35 days. The appearance of filariform larvae on the third day in the greater number of cultures will permit the deduction that the maximum life of larvae is 32 days. No evidence of lack of viability of larvae was observed as the result of serial transfer.

Of the 27 guinea pigs in this series, 21 were killed and given a thorough post-mortem examination, one was killed accidentally and received no autopsy, and five were used for other investigations. Nine of the 21 were killed to obtain adult *Strongyloides*, while the other 12 were killed some months after infection in order to check the negative faecal tests that were being recorded.

None of these 12 animals revealed any stages of *Strongyloides* in the lungs, heart, liver, small or large intestine. It is therefore considered that the infection of these animals was eliminated rather than that the parasitic females were becoming non-fecund.

Serial Infection of the Guinea Pig. Series II and III

Owing to the peculiarities observed in the free-living phase of Series I, two further series were commenced to repeat the observations under conditions as nearly identical as possible. The data obtained relating to longevity of free forms and length of life of cultures displayed such similarity to the results shown graphically for Series I that they did not seem of sufficient importance to warrant repetition.

Series II extended from July 1937 to June 1938, and during this period *S. agoutii* was serially transferred through 15 guinea pigs, 92 faecal cultures were prepared, and 919 observations were recorded. Filariform larvae used for infection purposes ranged from 3 to 18 days in age; in most cases, four-day-old larvae were used. Guinea pigs Nos. 1 and 2 were the only females in this series.

The mode of development was very inconsistent. Cultures from Nos. 1 and 3 yielded a few free females in addition to the filariform larvae and free males. Cultures from No. 7, however, gave filariform larvae and free females almost exclusively and in large numbers, though one or two males were observed. All other cultures produced filariform larvae and free males only; in a few cases, no males were observed. Since no constancy in mode of development was apparent, the series was discontinued after the fifteenth individual was infected. Of the 15 guinea pigs, six were killed by over-infection or to obtain adult parasites, six were killed after some long period to check reliability of faecal examinations, and three were used for further studies.

The studies in Series III consisted of the serial transfer of *S. agoutii* through 12 guinea pigs, from January to July, 1938, and included 60 cultures and 804 observations. The larvae used for infection purposes ranged from 3 to 12 days old, but were usually four days old.

The type of development in Series III was also variable. A few free females were recorded from cultures from guinea pigs Nos. 3 and 10. The remaining cultures tended to show direct development, with free males recorded intermittently throughout. Because of the variable results, the series was concluded after infection of No. 12. Eight of the 12 animals were killed or died as a result of over-infection. Adults were obtained from five only, the other three being negative. The remaining four animals were used for further study.

The Free-living Development of *S. agoutii* from the Agouti and Guinea Pig

In a previous paper by the author (10) on *S. agoutii* of the agouti, the indirect mode of development of the free-living generation was found to prevail. In this study, it has been shown that a mixed type appears in cultures of

faeces from guinea pigs infected with *S. agoutii*. This mixed type is represented by filariform larvae and free-living males, though free females have appeared. Two types of free-living development have thus been shown for this strain of *Strongyloides* in different hosts.

An attempt was made to ascertain whether the factors influencing the type of development could be attributed to the host or to the environment of the culture media. A young male guinea pig was infected percutaneously with *S. agoutii* from the agouti. Upon appearance of ova in its faeces, three groups of cultures were made: (i) faeces; (ii) ova removed from faeces by crushing, sieving, washing, and sedimentation, were seeded on guinea pig faeces from uninfected young stock and (iii) on sterile agouti faeces (autoclaved at 14 lb. pressure for 15 min.). Sterility of this agouti faeces, and also of faeces from the uninfected guinea pigs, was verified by control cultures.

To obviate error in culture examination, these were examined daily under the binocular microscope, and a sample was examined by the Baermann technique. When cultures yielded only filariform larvea or no larvae for five or six days, examinations were discontinued.

Of 10 cultures in group (i), all showed mixed development, as would be expected. In group (ii), 10 cultures all yielded filariform larvae and free males, with one free female in one culture. In group (iii), three out of four cultures showed the indirect mode of development, with one larva observed on the first day in one culture. A further 13 cultures in this group showed seven with indirect development, five with no development, and one with two larvae on the fourth day.

In the second and third groups, the population of the cultures was low in many cases. The uncontrolled process employed in seeding cultures with ova, which was not carried out quantitatively, may possibly explain this.

Discussion of Free-living Development of Strongyloides

In many instances when a species of *Strongyloides* is experimentally established in a new host of different taxonomic position from its usual or normal host, the life cycle is observed to be radically changed from the type manifested in the original host. Inadequate observations have on occasion led to the belief that a certain strain is exclusively direct or indirect, whereas actually few such strains appear to exist.

From the data obtained on the free-living phase of cultures from Series I, definite peculiarities in the mode of development were observed. By transfer of the agouti strain of *Strongyloides* through the guinea pig, a direct type of development might be expected. In these studies a modified direct mode was recorded, being represented by filariform larvae and free males. This mixed development was evidenced in cultures from the first 19 animals of the series, after which free females were recorded. Similar observations were recorded in Series II and III, though females occurred with considerable irregularity and inconsistency.

In an attempt to explain this phenomenon the current hypotheses will be reviewed as briefly as possible. Sandground (13) regards the parasitic female as syngonic, giving rise to ova which develop to males or females. If ova are fertilized in equal numbers, theoretically both sexes would be reproduced in equal numbers, but this seldom occurs. If only females or males are produced in the bi-sexual generation, he considers that the type of sperm that determines the mixing sex has either not been produced or has degenerated without functioning. Direct development might readily be attributed to parthenogenesis, but Sandground found the same syngonic condition occurred in parasites that were known to have been producing the direct type of development almost exclusively. However, the finding of sperm in his syngonic females does not completely exclude the occurrence of parthogenesis. Sandground further provides an explanation for direct development by consideration of the occurrence of an atypical distribution of the chromosomes at mitosis, which would constitute the determining mechanism for this mode. He suggests auto-infection as an explanation for direct development, on the hypothesis that in an infection originally showing both direct and indirect modes, the larvae of direct metamorphosis may, by auto-infection, reinforce the existing infection. Since larvae have been shown to develop mainly according to the parental history, it will follow that with the increasing age of an infection, the direct mode will finally predominate. Sandground (13) has observed in daily cultures of material from a rat, that within a short period, the sex-ratio may range from a bisexual generation almost entirely of females to another of males only. Following the revolutionary discovery of the parasitic male by Kries (12) and Faust (5), the latter proposed a possible explanation for alternative modes of development. He suggested that fertilized ova may give rise to an indirect mode of development, unfertilized to a direct mode. After the original supply of spermatozoa stored in the females becomes exhausted, the progeny of parasitic females (by parthenogenesis) in the intestine would be direct only, since the parasitic males are usually found in the bronchioles and consequently would not re-fertilize the females.

Following his investigation with *S. simiae*, Beach (1, 2) proposed the theory that under optimum conditions indirect development consistently occurred, whereas unfavourable conditions tended to modify the strain toward a direct type. Beach considers that the sex is established before or at the time of formation of the egg, but the development is modified by factors such as nutrition, viscosity, and toxicity. He further suggests that filariform larvae, appearing as a result of changing from an indirect to a mixed type are, in reality, potential sexual individuals. His work strongly indicates that directness and indirectness are conditioned by environment and not by genetic constitution. Graham (9), however, presents evidence indicating that the mode of larval development followed by *S. ratti* is determined prior to oviposition.

The occurrence of parthenogenesis in the first 19 experimental animals of Series I could readily explain direct development if no free males had been present. Chance hyperinfection of guinea pig No. 20 might have permitted fertilization of a parasitic female with subsequent production of indirect development. However, since the ova of *Strongyloides* are evacuated from the guinea pig in the late stages of segmentation (although usually embryonated in the agouti), it seems improbable that Faust's hyperinfection or Sandground's autoinfection would occur.

Parasitic males were not observed on any occasion, which supports the suggestion of Faust that unfertilized ova may produce a direct type of progeny. However, this fails to account for the females in the twentieth generation, and is inadequate to explain the free males throughout the series.

The conditions experienced by the *Strongyloides* strain in cultures of guinea pig faeces may have been sufficiently unfavourable to influence the strain to a direct mode of development. But as all guinea pigs received materially the same rations and all cultures were made under as nearly identical conditions as possible, this influence would be constant throughout the series.

Beach (2) considers that filariform larvae, produced when first-generation rhabditiform larvae are grown on artificial media, are potential free-living adults. He has demonstrated the alteration of a supposed indirect type of development of *S. simiae* to a mixed type when the worms were grown on artificial media. This mixed type showed large numbers of first-generation filariform larvae, a considerable number of males, and few or no females. On addition of small amounts of aqueous extracts of monkey faeces to the media, a more favourable growth was induced, and the mixed development tended to revert to the original mode, i.e., there was an increase in females and a decrease in the filariform larvae. These observations appear comparable with the data of the present study, and Beach's deductions are more readily applicable than any previously discussed. It seems logical to assume that indirect development is normal for *Strongyloides*, direct development occurring when unsuitable conditions are encountered. As a result of this adaptation, filariform larvae of the direct mode are considered as suppressed free females and males, which manifest their true forms only in the presence of certain definite requirements.

In these studies, the composition of guinea pig faeces apparently does not completely furnish the necessary medium for the appearance of free females and males with regularity, though males have appeared more consistently than females.

The results obtained from the culture of ova of *S. agoutii* from the guinea pig on sterile agouti and guinea pig faeces present strong evidence in support of the theory that directness and indirectness are conditioned by environment. These two cultures of ova displayed different modes of free-living development. The former reverted to the original indirect type of the agouti strain; the latter showed the development of free males and filariform larvae, as usually observed in cultures from a guinea pig infected with *S. agoutii*.

A number of cultures of agouti faeces seeded with ova from guinea pig faeces showed no development. This condition may probably be attributed to the uncontrolled method of distribution of ova on cultures or to some unknown factor preventing development of the ova.

The mechanical methods employed in obtaining these ova did not affect the mode of development of larvae. From the results obtained it is assumed that the factor controlling the mode of free-living development of *S. agoutii* is directly associated either physically or chemically with the media in which the eggs are cultured.

The Prepatent and Patent Periods of *Strongyloides agoutii* in the Guinea Pig

The prepatent and patent periods in members of the genus *Strongyloides* have been variously determined and have shown considerable diversity for different species and strains. Sandground (14) reported the prepatent period of *S. stercoralis* to be 5 to 10 days in dogs and 9 to 16 in cats. Faust *et al.* (7) recorded infections of dogs with human strains of *S. stercoralis* in which the prepatent period usually ranged from 11 to 18 days. Graham (8), using single larva infection of rats with *S. ratti*, showed the prepatent period to range from 5 to 11 days, most individuals being positive by culture on the sixth day, whereas Sheldon (15) recorded the prepatent period of *S. ratti* as ranging from 3 to 6 days.

In these studies, the three series of guinea pigs infested with the agouti strain of *Strongyloides*, together with a few other individuals employed in immunity investigations, gave considerable data on the period between infection and the appearance of ova in the faeces. All animals were tested on the sixth day after infection and daily thereafter. The frequency distribution of the first day of appearance of ova, after exposure to filariform larvae, is recorded in Fig. 4. In spite of the many factors that may impede the migration of larvae to the intestine or delay the discharge of eggs, it is of interest to note the narrow range to which the first appearance of ova is confined. Of the 58 animals, 24% were positive by faecal test on the seventh day, 47% on the eighth, 22% on the ninth, and 7% on the tenth day; that is, 71% were positive by the eighth day, the modal point for the series.

These observations show the commencement of the patent period to be confined to comparatively narrow limits; in fact, these limits are considerably narrower than those at the end of this period. The patent periods reported for the genus *Strongyloides* are found to show considerable variation. Sandground (14) has found the duration of *S. stercoralis* infection to be from 2 to 11 months in dogs and from 2 to 7 months in cats. Faust *et al.* (7) state that infection of the dog with human strains of *Strongyloides* is maintained for only a period of weeks or months, as contrasted with a period of years in man, the natural host. The patent period of *S. ratti* has been shown by Sheldon (15) to range from 51 to 136 days. In the present studies with *Strongyloides*, the period over which eggs have been passed in the faeces has been found to show

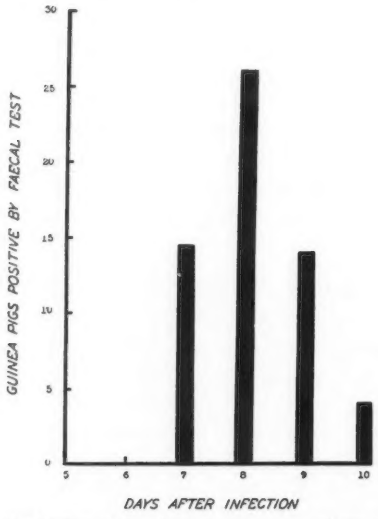

FIG. 4. *Frequency distribution of first day of appearance of ova in faeces of 58 guinea pigs after infection with S. agoutii.*

considerable variation. Eggs were recorded in the faeces of 26 guinea pigs for three to eight weeks. The majority of infections terminated during the fourth or fifth week. A number of individuals were killed subsequent to a negative faecal test, and autopsy showed that the test was a true indication that the infection had disappeared rather than that the females become non-fecund. As the animals were infected with filariform larvae en masse, no data were available to show if the heavier infections were lost more quickly than the lighter ones. It was observed, however, that the older the host at the time of infection, the earlier the infection was lost.

Observations on Migration of Larvae of *S. agoutii* Through the Guinea Pig

It is generally agreed that, following percutaneous infection, the path of migration of *Strongyloides* necessitates the passage of the larvae through the lungs prior to their establishment in the small intestine. To ascertain the rate of migration and to observe the organs in which the various stages were found, four guinea pigs were infected and killed at intervals over a period of eight days.

The animals employed were young males, six weeks old at time of exposure. They were infected en masse with *S. agoutii*, as previously described. Autopsies were made and all suitable organs examined by the Baermann technique to obtain any migrating larvae. In each case, the autopsy included the examination of the trachea, lungs, liver, heart, spleen, kidneys, stomach, small and large intestines.

The first guinea pig was killed 48 hr. after exposure and one filariform larva was obtained in the heart. The second was killed after 72 hr.; a few larvae were observed from the lungs, heart, stomach, duodenum, and jejunum. The third was killed after 96 hr. and yielded filariform larvae from the heart and stomach; larvae in stages of metamorphosis between the filariform larva and the adult were found in the duodenum and jejunum. The fourth was killed on the eighth day, and one filariform larva was obtained from the heart; the duodenum and jejunum yielded large number of both immature and ovigerous females, and a few were also found in the ileum.

As a result of these infections, it appears that with this species the migration through the lungs is of a transitory nature; no forms other than filariform larvae were observed in that organ. The time of migration through the blood stream extends over a considerable period of time, since a larva was collected from the heart as late as the eighth day subsequent to infection.

Acquired and Age Resistance of the Guinea Pig to Infection with *S. agoutii*

The subject of resistance and immunity of the host to infection with helminths has received but little attention until recent years. From the point of view of the host there are three major types of resistance mechanism. The first type is natural immunity, which is found frequently among animals. Most individuals are found to be naturally immune to parasites of unrelated animals, though wide adaptations are known to exist. The second type is that of age resistance. This is displayed by animals that may be susceptible in early life to infection with a parasite, but become less susceptible later. The third type is that which is acquired by the host as a direct consequence of infection with a specific parasite.

Previous work on resistance to re-infection among members of the genus *Strongyloides* is scanty, and Sandground (14) appears to have been the first to make studies of this nature. He reported investigations on susceptibility, resistance, and acquired immunity of dogs and cats to infection with *S. stercoralis*. Successful infection of dogs and cats of all ages was brought about with a human strain of *S. stercoralis*, and animals that lost their infection were found to be refractory to re-infection; the acquired immunity in dogs lasted for more than six months. It was concluded that in immune animals the larvae reach the intestine but do not attain sexual maturity. Kotlan and Vajda (11) report an age immunity to *S. ransomi* occurs in pigs. Sheldon (16) carried out quantitative studies on acquired resistance and showed that a marked resistance was acquired by rats as the result of super-infection with *S. ratti*. He also demonstrated that resistance was acquired and retained by rats as the result of a single previous infection that had run its course and disappeared; they were also found to be successfully immunized by serial injections of heat-killed larvae in a saline suspension. Animals two or four months old were found to be equally susceptible to infection with *S. ratti*;

at eight months resistance was observed, at 12 months was less apparent, and was observed again at 17 months.

A series of experiments was undertaken to determine if an acquired or an age resistance was manifested by guinea pigs to *S. agoutii*. If an acquired resistance was exhibited, it was hoped to discover whether this tended to pass off after a time or if it inhibited reproduction of the female or prevented the parasite from establishing itself in the intestine with the resultant expulsion of the worm in the faeces.

A total of 17 guinea pigs was used. Several of these had been used in the experiments on serial transfer, thus providing excellent material on which to attempt re-infection with the same parasite. Infection of the guinea pigs was effected percutaneously, as described previously. Diagnosis of infection was determined by faecal test.

The age of animals employed in these tests varied to a considerable extent. Control guinea pigs were employed to check the infectivity of larvae used for re-infection trials. Post-mortem examinations were not made on all guinea pigs as some were used for other purposes. In certain instances when negative faecal tests were recorded, autopsy was carried out to ascertain if females were present but had been rendered non-fecund by an immunity mechanism. In no case was this found to occur.

The essential data relating to this series of infections are presented in the accompanying tables. To determine if any resistance existed, a preliminary trial was initiated, the results of which are presented in Table I. Three guinea pigs had previously been infected with *Strongyloides*. Prior to the second exposure they were shown by faecal test to have eliminated their primary infection. This exposure was about six months after the first; they did not become re-infected. A third exposure was made one month after the second but no evidence of infection was observed. No autopsy was carried out.

The second group comprised four individuals previously infected and two controls (Table II). These four animals gave a negative faecal test for several months before re-infection was attempted. Since guinea pig No. 4 was exposed to larvae from a different culture to that used as a source of larvae for Nos. 5, 6 and 7, two young controls were exposed to ensure the infectivity of both lots of larvae. The guinea pigs were all exposed to infection on the same day, and subsequent faecal tests yielded negative results for Nos. 4–7; both controls were positive. Chandler (4) has shown that a resistance acquired as a result of a previous infection of *Nippostrongylus muris* in rats may materially interfere with reproduction of the parasite and cause a pronounced reduction in egg output. In view of this fact it was considered advisable to autopsy Nos. 4–7, to make sure that the routine faecal tests were not yielding inaccurate results. Nos. 5 and 6 were autopsied 14 and 13 days after infection, Nos. 4 and 7, 31 days after infection. Thorough examination of the lungs, heart, liver, small and large intestines did not yield any stages of *Strongyloides*. The control to No. 4 was killed and examined on the 23rd

TABLE I

PRELIMINARY TRIAL OF EXPOSURE OF GUINEA PIGS TO RE-INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., months	Date of 1st expos., 1937	Faecal tests after 1st exposure		Date of 2nd expos., 1938	Faecal tests after 2nd exposure		Date of 3rd expos., 1938	Faecal tests after 3rd exposure	
			Date	Result		Date	Result		Date	Result
1	4½	June 17	June 25, 26, 28, 29 July 15 Aug. 16 Oct. 13, 27 Dec. 1, 21 Jan. 1	Positive Positive Positive Negative Negative Negative	Jan. 27	Feb. 3–5, 9–11, 15, 22, 25, 28	Negative	Mar. 2	Mar. 9–12, 14, 15, 22	Negative
2	5	July 15	July 24, 26, 27 Aug. 5, 16 Oct. 10, 26 Dec. 1, 21 Jan. 12	Positive Positive Negative Negative Negative	Jan. 27	Feb. 3–5, 9–11, 15, 17, 19, 22, 25	Negative	Mar. 2	Mar. 9–12, 14, 15, 22	Negative
3	2	July 27	Aug. 5, 6, 16 Oct. 13, 19, 27 Dec. 12 Jan. 1	Positive Negative Negative Negative	Feb. 2	Feb. 9, 10, 15, 17, 20, 22, 25, 28	Negative	Mar. 2	Mar. 10–12, 14, 15, 24	Negative

TABLE II

EXPOSURE OF GUINEA PIGS TO RE-INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., months	Date of 1st expos., 1938	Faecal tests after 1st exposure		Date of 2nd expos., 1938	Faecal tests after 2nd exposure	
			Date	Results		Date	Results
4	12	April 1	April 9–11, 13, 19, 27 May 16, June 6, 20 July 18, Aug. 9, Oct. 13, 17, 24	Positive Negative Negative	Dec. 5	Dec. 12–17, 19, 20, 27	Negative
Control	1½	Dec. 5	Dec. 12–17, 19, 20	Positive			
5	2	Jan. 10	Jan. 19, 20, Feb. 3, 17 Feb. 22, 28, Mar. 22 April 4, 19, May 16 June 6, 20, July 18 Aug. 9, Oct. 13, 17, 24	Positive Negative Negative Negative Negative	Dec. 5	Dec. 12, 14, 15, 17, 18	Negative
6	2	Mar. 10	Mar. 17, 18, April 5 April 19, May 5 June 6, 20, July 18 Aug. 8, Oct. 13, 17, 20	Positive Negative Negative Negative	Dec. 5	Dec. 12–16	Negative
7	2	Mar. 29	April 4, 6, 19, May 16 June 6, 20, July 18 Aug. 9, Oct. 10, 17, 24	Positive Negative Negative	Dec. 5	Dec. 12–20, 27	Negative
Control	2	Dec. 5	Dec. 14–17, 19	Positive			

POST-MORTEM EXAMINATION

No. 4. Killed Jan. 5, 1938. All organs (lungs, heart, liver, duodenum, jejunum, ileum, large intestine) negative for *Strongyloides*.
Control. Killed Dec. 28, 1938. Small intestine heavily infected with adult *Strongyloides*.
No. 5. Killed Dec. 19, 1938. All organs negative for *Strongyloides*.
No. 6. Killed Dec. 18, 1938. All organs negative for *Strongyloides*.
No. 7. Killed Jan. 5, 1939. All organs negative for *Strongyloides*.

day and displayed a heavy infection of *Strongyloides* in the small intestine; the other control guinea pig was used for further experimentation.

To check these results another experiment was undertaken in which three previously infected, and two control, animals were employed. The essential data on this trial was summarized in Table III. Before attempting infection for the second time, the guinea pigs were shown to have been negative to faecal tests over a period of some months. One control was employed to test the infectivity of larvae used for application to Nos. 8 and 9, another for No. 10. After attempting infection, these animals received the usual routine faecal examinations, negative results being recorded in all cases. Nos. 8 and 9 were autopsied on the 9th and 11th days after exposure; the control was killed on the 8th day. No autopsy of No. 10 and the control was carried out since they were held for further use. Post-mortem examination of Nos. 8 and 9 did not reveal any stages of *Strongyloides* in the intestine, whereas one larva was recorded from the heart of the control, and the small intestine was heavily infected with adults.

TABLE III

EXPOSURE OF GUINEA PIGS TO RE-INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., months	Date of 1st expos.	Faecal tests after 1st exposure		Date of 2nd expos., 1939	(F) Faecal and (B) Baermann* tests after 2nd exposure	
			Date	Results		Date	Results
8	2	Dec. 20 1937	Dec. 30, Jan. 17	Positive	Jan. 9	(F) Jan. 16–18	Negative
			Feb. 3, 20, 22, Mar. 15, 22	Negative		(B) Jan. 10–18	Negative
			April 5, 19, May 16	Negative			
			June 6, 20, July 18	Negative			
			Oct. 13, 17, 24	Negative			
9	2	May 13 1938	May 20–22, June 18, 20	Positive	Jan. 9	(F) Jan. 16–20	Negative
			Aug. 8, Oct. 13, 17, 25	Negative		(B) Jan. 10–20	Negative
Control	1½	Jan. 9 1939	Jan 16	Positive	Jan. 9		
10	2	June 2 1938	June 10, 13, 20, July 18	Positive	Jan. 30	(F) Feb. 7, 9–11, 13–15, 20, 23	Negative
			Aug. 9, Oct. 13, 17, 25	Negative		(B) Jan. 31–Feb. 8	Negative
Control	1½	Jan. 30 1939	Feb. 7–9, 10, 13, 20, 27	Positive			
			Mar. 21	Negative			

* *Baermann examination was made in morning and late afternoon each day.*

POST-MORTEM EXAMINATION

No. 8. Killed Jan. 18, 1939. All organs negative for *Strongyloides*.
No. 9. Killed Jan. 20, 1939. All organs negative for *Strongyloides*.
Control. Killed Jan. 17, 1939. 1 larva in heart. Intestine heavily infected with adult *Strongyloides*.

From Table III it will be seen that for the first 8 to 10 days following the second exposure to infective larva, a Baermann examination was introduced in addition to the routine faecal test. When an acquired resistance is manifested by an individual, there appears to be a generalized condition which may inhibit development to the adult stage or prevent successful establishment of the invading parasite within the intestine. In view of this fact and on the assumption that the migrating infective larvae would, if they arrived at the intestine, still be active, collection of the total faecal output of Nos. 8, 9, and 10 was made twice daily and Baermanned, the sediment being drawn off after one-half and one hour. It was anticipated that if the migrating larvae did not become established in the intestine they would be passed out, but no larvae were found.

Since a number of the individuals employed in the preceding trials were, on an average, about one year old at time of re-exposure, an attempt was made to determine whether this resistance was due to an age factor. Three guinea pigs known to be considerably more than a year old were used for this purpose, the data on which are recorded in Table IV. All three were

TABLE IV

INFECTION OF ADULT GUINEA PIGS WITH INFECTIVE LARVAE OF *Strongyloides agoutii*

Guinea pig	Date exposed, 1938	Faecal tests	
		Date	Results
A	Dec. 29	Jan. 7–10, 12, 16, 18, 20, 23, 25, 30 Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative
B	Dec. 29	Jan. 7–10, 12, 16, 20, 23, 25 Jan. 30, Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative
C	Dec. 29	Jan. 7–10, 12, 16, 18, 20, 23 Jan. 25, 30, Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative

infected on the same day and faecal tests throughout the month following exposure were all positive. The infection was observed to pass off after the first month; these animals were not autopsied but were held for further use.

The preceding experiments show that the guinea pig acquires a resistance to re-infection with *Strongyloides*. This resistance does not permit the establishment of the parasite in the intestine, and animals that have lost their infections cannot be re-infected over a period ranging at least from 6 to 13 months after the primary infection. An attempt was made to ascertain whether infective larvae were expelled from the intestine with the faecal material; results of observations were negative but because of complicating factors these observations are not considered conclusive.

Acknowledgments

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THE HELMINTH PARASITES OF SLEDGE-DOGS IN NORTHERN CANADA AND NEWFOUNDLAND[1]

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Abstract

In a survey, based on examination of faeces and viscera of sledge dogs, the following were identified: hookworms, ascarids, whipworms, kidney worms, fish-carried and other tapeworms, the Canadian liver-fluke and other trematodes and an acanthocephalid. The distribution of the infections is noted. The acanthocephalid, *Corynosoma semerme*, is recorded for the first time from North America.

Since 1933, the Institute of Parasitology has been engaged in a survey of the parasites of animals in Canada. During that period it has accumulated a considerable amount of data on infections of economically important animals in the Arctic and sub-Arctic regions.

The parasitic fauna of animals is of considerable importance to the inhabitants of these regions. The aboriginal peoples live entirely on the animals that breed there and although of an advanced culture, many, particularly Esquimaux, eat a large part of their meat and fish uncooked. Because of the rigour of the climate, human beings and domestic animals live close together and make the transmission of helminths a simple matter. Because of the severe climate, bacterial infections are somewhat rare and animal parasites have become of even greater significance.

The animal of greatest importance is the dog. Transportation and communication of white man and native, alike, depend almost exclusively upon this animal. Consequently an investigation of the parasites of this animal has been a first consideration. The data at our command are now sufficient to record and give an adequate picture of the geographical distribution of its parasites in these regions.

The information was mostly obtained through co-operation of the R.C.M. Police, the National Parks Branch, and the Hudson's Bay Company in Canada, and of the Newfoundland Rangers in Labrador. In addition, both junior authors have personally collected in the eastern Arctic, one (I.W.P.) in 1933 in the north and west sides of the Quebec Peninsula, and the other (L.L.L.) in 1939 from that region and from Baffin, Ellesmere, Southampton and Somerset Islands, and the west coast of Hudson's Bay.

Much information has been obtained by examination of faeces sent to the Institute by interested persons, including the R.C.M. Police, Game Wardens,

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Newfoundland Rangers, and others. In most cases this material was unpreserved, and, although sometimes several months elapsed before it was delivered in Montreal, virtually all arrived in good condition. On a few occasions it was formalized. Most faecal material was shipped in two ounce tin ointment boxes, and all was examined by simple, repeated concentration. These examinations and identifications were made by the senior author.

In addition, dog carcasses or entrails have been sent to the Institute or examined on the spot. Those sent in (many coming in the "Nascopie") were formalized and shipped in a semi-mumified condition (e.g., superficially dry) in garbage cans. These cans were packed with moist material to prevent undue drying and in most cases the technique functioned adequately.

FIG. 1

In no instance was a parasite-free collecting point recorded although certain parasites have quite definite geographical ranges. The distribution is considered by species.

Nematodes

Hookworms

Hookworms have been found in dogs in all areas surveyed and they occur in the most northerly station in North America, viz., Craig Harbour, Ellesmere Island, N.W.T. In many cases, the diagnosis has been based only on eggs. As, however, only the northern dog hookworm *(Uncinaria stenocephala)* has been found (on autopsy) north of Moosonee, it is probable that all these records refer to this species.

Egg records are as follows—

Arctic Red River, Mackenzie District, N.W.T.
Battle Harbour, Labrador.
Bonne Baie, Nfld.
Cambridge Bay, Victoria Island, N.W.T.
Cartwright, Labrador.
Chimo, Ungava Bay, Que.
Craig Harbour, Ellesmere Island, N.W.T.
Flower Cove, Nfld.
Fort Fitzgerald, Alta.
Fort George, Man.
Hebron, Labrador.
Hopedale, Labrador.
Lake Harbour, Baffin Island, N.W.T.
Maitland Point, Mackenzie District, N.W.T.
McLeod Lake, B.C.
Moosonee, James Bay, Ont.
Nain, Labrador.
Northwest River, Labrador.
Pangnirtung, Baffin Island, N.W.T.
Pine River, Man.
Port Burwell, Hudson Strait, N.W.T.
Port Harrison, Hudson Bay, Que.
Stupart Bay, Hudson Strait, Que.
William Harbour, Labrador.
Wood Buffalo Park, Mackenzie District, N.W.T.

In addition, this species has been found in carcasses from Dundas, Moosonee, Burwell, Sept Isles, and Nipigon. Two dogs were positive at Dundas; of these one was born on Devon Island while the other had been imported from Pangnirtung on Baffin Island. This species also occurs in dogs on the Island of Montreal, P.Q., and is common in foxes throughout Eastern Canada.

Ancylostoma caninum, the southern dog hookworm, was found in one dog from Moosonee, in association with the northern species. This appears to be the most northerly record of its occurrence. It also occurs on the Island of Montreal, P.Q.

Ascarids

Ascarids are almost equally widely spread, the predominant species being *Toxascaris leonina* (syn. *T. felis*) although *Belascaris marginata* (syn. *Toxocara canis*) also occurs; these species were not separated in the writers' egg records, although most were *T. leonina*.

Egg records were obtained from:

Cartwright, Labrador.
Chimo, Ungava Bay, Que.

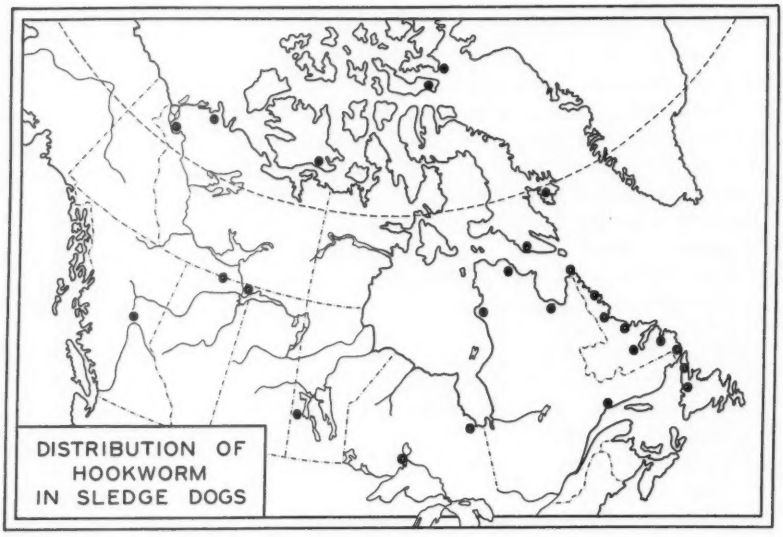

FIG. 2

Churchill, Man.
Cumberland House, Sask.
Eskimo Point, Keewatin District, N.W.T.
Fort Chipewayan, Alta.
Fort Resolution, Mackenzie District, N.W.T.
Hebron, Labrador.
Hopedale, Labrador.
Lake Harbour, Baffin Island, N.W.T.
Maitland Point, Mackenzie District, N.W.T.
Moosonee, James Bay, Ont.
Nain, Labrador.
Rupert House, James Bay, Que.
William Harbour, Labrador.
Wood Buffalo Park, Mackenzie District, N.W.T.

In addition, actual specimens of *T. leonina* from dogs were collected from Cape Smith, Dorset, Moosonee, Nottingham, Stupart and Wolstenholme, and of *B. marginata* from Moosonee and Wolstenholme.

Whipworms

Whipworms (*Trichuris vulpis*) were seen on only one occasion (Kenora, Ont.) and were not recovered from Arctic regions at all. They are fairly common in southern Canada.

Kidney Worm

A single infection of *Dioctophyme renale* was recorded from Berens. In this case both kidneys were infected and the female worms reached a length of 30 in. The dog died as a result of this infection.

Tapeworms

Diphyllobothrium spp. At least two species of this genus appear to occur in dogs in the north of Canada. One, which is found in the Mackenzie River basin, is associated with fresh-water fish and is almost certainly *D. latum*. Most records were based on eggs in the faeces, but actual tapeworms found in man in northern Saskatchewan belong to this species, and the distribution is continuous from this region.

The other species, occurring in northern Quebec and along the Arctic coast, has a longer egg and the carrier fish may be assumed to be a salt-water one. The few specimens recovered from dogs in that region were in too poor a state of preservation for proper identification and could be referred neither to an existing species nor a new one. Accordingly, while the writers believe that at least two species exist, they have not attempted, at this stage, to identify them by name.

Eggs of tapeworms of this genus were found in the following places:

Arctic Bay, Baffin Island, N.W.T.
Baillie Island, N.W.T.
Berens, Man.

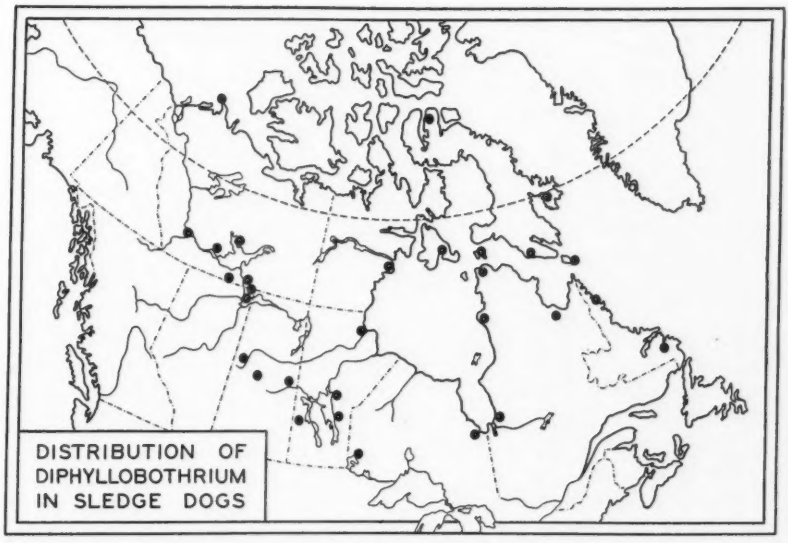

FIG. 3

Cameron Bay, Keewatin District, N.W.T.
Cartwright, Labrador.
Cat Lake, Ont.
Chesterfield, Keewatin District, N.W.T.
Chimo, Ungava Bay, Que.
Churchill, Man.
Coral Harbour, Southampton Island, N.W.T.
Cumberland House, Man.
Forteau, Labrador.
Fort Chipewayan, Alta.
Fort Fitzgerald, Alta.
Fort Providence, Mackenzie District, N.W.T.
Fort Smith, Mackenzie District, N.W.T.
Goldfields, Man.
Hebron, Labrador.
Ile a la Crosse, Sask.
Kenora, Ont.
Lake Harbour, Baffin Island, N.W.T.
Moosonee, James Bay, Ont.
Norway House, Man.
Pangnirtung, Baffin Island, N.W.T.
Pine River, Man.
Port Harrison, Hudson Bay, Que.
Rae, Mackenzie District, N.W.T.
Resolution, Hudson Strait, Que.
Rupert House, James Bay.
Simpson, Mackenzie District, N.W.T.
Waskesiu Lake, Sask.
William Harbour, Labrador
Wood Buffalo Park, Mackenzie District, N.W.T.

Actual specimens referable to this genus were found in Wolstenholme and Nottingham Island.

Taenia spp.

Five dogs showed *Taenia* eggs in their faeces, but again it was impossible to refer them to a species. These cases came from:

Cameron Bay, Keewatin, N.W.T.
Churchill, Man.
MacLeod Lake, B.C.
Pine River, Ont.
Rupert House, James Bay, P.Q.

Specimens of *Taenia*, not identifiable because of partial decomposition, were found in Notre Dame du Nord, Que., and Cape Smith.

Dipylidium caninum

This tapeworm was found in carcasses from Moosonee, Nottingham and Wolstenholme.

Trematodes

Metorchis conjunctus

The Canadian liver fluke was found in dogs from:

Berens, Man.
Cat Lake, Ont.
Cumberland House, Sask.
Ile a la Cross, Sask.
Kenora, Ont.
Lac la Ronge, Sask.
Lake Mistassini, Que.
Moosonee, James Bay, Ont.
Norway House, Man.
Pine River, Ont.
Rupert House, James Bay, Que.
Trout Lake, Ont.
Waskesiu Lake, Sask.

The southern limit of this parasite appears, from present records, to be the St. Lawrence and the Canadian border. Its northern limit in Quebec is unknown, but its western and northern limits appear to be the height of land in Saskatchewan and the borders of the N.W.T.

Cryptocotyle lingua

This intestinal trematode, which appears to have been introduced to the Maritime Provinces of Canada, has spread both north and south. It is now found in the St. Lawrence estuary and the Labrador.

Records from the area under review include:

Anticosti Island, Gulf of St. Lawrence.
Cartwright, Labrador.
Flower Cove, Nfld.
Hebron, Labrador.
Hopedale, Labrador.

Alaria sp.

A species of Strigeid, which on the morphology of the egg and its resemblance to that found by us in actual trematodes, we refer to this genus, is recorded from:

Cumberland House, Sask.
Moosonee, James Bay, Ont.
Rupert House, James Bay, Que.
Wood Buffalo Park, Mackenzie District, N.W.T.

Actual specimens of a species of *Alaria* were recovered from a dog from Moosonee.

Miscellaneous Trematodes

Trematode eggs, which the writers were unable to identify because of their non-correspondence to published descriptions or to specimens collected from these regions, occurred in the faeces of dogs from:

Berens, Man.
Cambridge Bay, N.W.T.
Eskimo Point, N.W.T.
Fort Smith, N.W.T.
Port Harrison, Que.

Acanthocephala

A single species of Acanthocephala was recovered from a husky dog at Dundas, N.W.T. It has been identified by Prof. H. J. Van Cleave as *Corynosoma semerme* (personal communication), a species hitherto unrecorded from North America.

A NEW TREMATODE, *FIBRICOLA LARUEI*, FROM THE RACOON IN CANADA[1]

By M. J. Miller[2]

Abstract

A new trematode, *Fibricola laruei*, is described from the racoon in Canada.

The racoon (*Procyon lotor*) in the Province of Quebec was found to harbour an undescribed strigeid of the sub-family Alariinae. Its morphology showed it to be most closely related to members of the genus *Fibricola*, to which it is assigned, with the specific designation *laruei* sp. nov.

Description

Small, comparatively thin forms measuring from 0.7 to 1.17 mm. in length when mature; body divided into two parts, the lengths of the anterior and posterior parts being in the approximate ratio of 3 : 2; anterior part of body flattened with the edges inrolled ventrally and meeting medially near the junction of the two segments of the body (0.46 to 0.5 mm. wide); posterior segment narrow and cylindriform (0.22 to 0.3 mm. wide); terminal oral sucker slightly longer than broad (average size, 0.076 by 0.066 mm.); pharynx conspicuous, elongate, about the same length as the oral sucker and about two-thirds as broad; oesophagus slightly shorter than pharynx; intestinal caeca extend well into the posterior segment; ventral sucker usually broader than long, situated at the junction of the anterior and middle thirds of the body (average size, 0.026 by 0.033 mm.); muscular holdfast organ situated medially at the posterior end of the anterior segment (average size, 0.11 by 0.07 mm.); vitellaria composed of numerous small follicles confined to the anterior segment; testes two in number situated asymmetrically in the posterior segment, the posterior testis considerably broader, reniform in shape, the anterior testis situated immediately above the posterior one usually on the right, but occasionally on the left side of the body, roughly spherical in shape (average size posterior testis, 0.06 by 0.17 mm.; average diameter anterior testis, 0.13 mm.) ovary small, wider than long, above and partially overlapping anterior testis in a dorsal position (average size, 0.08 by 0.05 mm.); eggs large, rarely over two present in the uterus at one time (average size, 0.12 by 0.07 mm.).

Discussion

According to Dubois (2) the subfamily Alariinae contains five genera: *Alaria*, *Cynodiplostomum*, *Fibricola*, *Pharyngostomum*, and *Podospathalium*. The present form obviously does not belong to either *Pharyngostomum* or

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada, awarded through the National Committee on Fish Culture.

[2] *Research Assistant.*

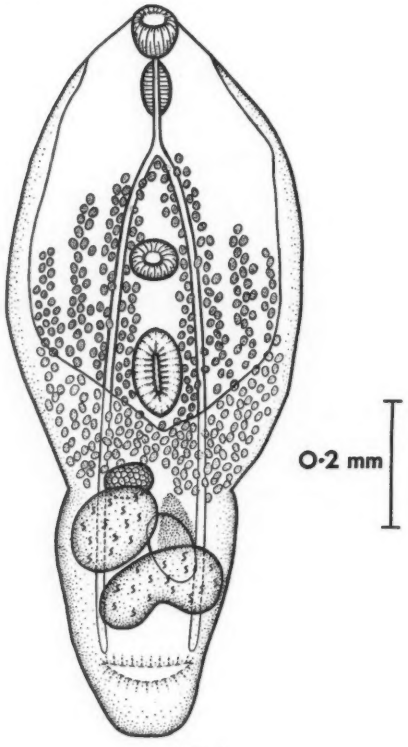

FIG. 1

Podospathalium. It differs from *Alaria* and *Cynodiplostomum* in that it has no pseudo-suckers or other appendages of any sort at the cephalic extremity. It differs from *Fibricola* in the character of the holdfast organ, which is elliptical in the present form and circular in *Fibricola*. With the exception of this character, however, its general morphology agrees very closely with that of the *Fibricola* species. The difference in shape of the holdfast organ does not appear to be sufficient reason for the creation of a new genus, and so the present form is assigned to the genus *Fibricola* and the generic definition amended to read as follows:

Alariinae with the body more or less distinctly divided into two parts; the cephalic extremity without sucker-like or other appendages; the holdfast organ either circular and one-third of the length of the anterior segment or elliptical and about one-fifth the length of the anterior segment; the posterior segment shorter than the anterior one; the ovary situated at the junction of the two segments; the posterior testis larger than the anterior one, which is asymmetrically developed opposite the Mhelis gland; no genital cone; shallow *burs copulatrix* with a subterminal pore.

Host: Racoon (*Procyon lotor*)

Location: Small intestine.

Locality: Argenteuil Co., Quebec, Canada. (It has also been found in racoons from other parts of Quebec.)

Types and paratypes in the helminthological collection, Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada.

References

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2. DUBOIS, G. Mem. Soc. Neuchateloise Sci. Nat. 6 : 535 pp. 1938.

A REVISION OF THE AMERICAN SPECIES OF *GONIA* MEIGEN (DIPTERA: TACHINIDAE)[1]

By Frank O. Morrison[2]

Abstract

Twenty American species of *Gonia* are separated on the basis of the shape of the anal forceps coupled with other characters. Four species, *G. grandipulvilli*, *G. discalis*, *G. albagenae*, and *G. tenuiforceps*, are described as new. The anal forceps are figured for each species. *G. brevipulvilli* Tothill is a synonym for *G. longiforceps* Tothill. A phylogenetic order and grouping of the species is based on the shape of the forceps, season of capture, and habitat. Complete taxonomic history of all categories is given.

Introduction

The tachinid genus, *Gonia* Meigen, has long been known to contain several distinct American species. Descriptions of at least 19 of these have been published. Determinations have been based on many different characters, of which those of the male genitalia have been favoured recently. It is, however, admittedly difficult, if not almost impossible, to describe in words slight differences in the shape or contour of such structures. Yet in no case has one of these characters been illustrated. As a result the accurate specific determination of *Gonia* has been practically impossible, and in almost all dipterous collections various species of this genus may be found under the name of *Gonia capitata* DeGeer, a European species of which the occurrence in America is very doubtful.

The recent extensive work on biological control has brought into prominence our various entomophagous parasites, and especially those parasitic on pests of economic importance. *Gonia* is known to be parasitic on lepidopterous larvae and has been held (17) to be an important control factor in damaging cutworm outbreaks in the grain-growing areas of Western Canada.

Townsend (21), discussing the habits of this and related insects that lay their microtype eggs on plant foliage, where they are ingested with the foliage by lepidopterous and other larvae, goes so far as to suggest that such parasites might be bred and distributed as a control measure for certain pests.

A clarification of the taxonomy of this group has been attempted in the present work by a study of the genital and other significant distinguishing characters. Where possible the work has been based on an examination of type material. A complete taxonomic bibliography of the genus and each species dealt with is included. Some synonomy is suggested, but it seems possible that, when further type material is available and especially when

[1] *Manuscript received January 19, 1940, and as revised May 9, 1940.*

Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Macdonald College Journal Series No. 138. Based on a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] *Lecturer, Department of Entomology, Macdonald College.*

European forms can be studied, further synonomy may be shown. Four new species are described and figured.

In approaching this problem an anatomical study of the male genitalia of *Gonia* was found imperative. Such a study forms the second part of this paper, to be published later. It was hoped that less easily exposed structures than those previously used might prove valuable in taxonomic work. Although no characters of taxonomic value were found, it became evident from the literature on the morphology of the male genitalia of Calyptrate Diptera that, though extensively used for taxonomy, the morphology of these structures is as yet poorly understood. The literature was carefully reviewed and synopsized. Considerations of space, however, make it inadvisable to include the synopsis. A table of synonomous morphological terms compiled from the literature is included, as are also the citations of the papers used and a detailed account of the anatomical study of the genital structures in *Gonia*, with some suggestions as to the possible homologies of the parts with those in other Diptera and with the parts of the male genitalia of more generalized insects. The taxonomy of Diptera in general cannot but be furthered by a more complete understanding of, and more uniform terminology for, the parts of these much used structures.

Historical Review

The genus *Gonia* was established by Meigen (9) in 1803, who later described 13 species (10). Curtis (5) designated *Musca capitata* DeGeer as the type of the genus. Desvoidy (6) described two genera, *Rhedia* and *Reaumuria*, which were synonymized with *Gonia* by Coquillet (3). Desvoidy had wrongly supposed the term *Gonia* preoccupied in conchology. Two other generic descriptions published by Desvoidy (7), *Isomerya* and *Pissemya*, have been synonymized with *Gonia* Meigen by Schiner (14) and Brauer and Bergenstam (2) respectively. Townsend (21), in his as yet only partly published "Manual of Myiology", recognizes as separate genera, *Reaumuria* Desvoidy (type *Gonia ornata* Meigen) and *Rhedia* Desvoidy (type *Gonia atra* Meigen), as well as three American genera: *Cnephalogonia* Townsend (type *Gonia distincta* Smith), *Cystogonia* Townsend (type *Gonia turgida* Coquillet) and *Knabia* Townsend (type *Knabia hirsuta* Townsend), and at least one other exotic genus, *Phosphocephalops* Townsend (type *Gonia pallens* Williston), all of which would fall within *Gonia* Meigen as considered here. *Isomerya* Desvoidy he synonymized with *Gonia* Meigen s.s. and *Pissemya* Desvoidy with *Rhedia* Desvoidy. It is impossible, however, to determine from the volumes published to date the limits of Townsend's restricted genera or their synonomy with species considered in this paper. Further definition of these forms must await the publication of the additional volumes of his work containing the generic and specific descriptions.

Meigen and other European taxonomists depended largely on colour characters and the relative lengths of the aristal segments for specific determinations in this genus. The resulting descriptions are totally inadequate to allow any

comparison of our now known American species with the European forms on the basis of these descriptions alone.

Say (13) described the first known American species, *Gonia frontosa* Say, stressing colour. What is probably the same species was redescribed as *Gonia philadelphica* Macquart (8) and again as *Gonia albifrons* Walker (22). Williston (24) recognized the synonym of the above mentioned names, redescribed the species as *Gonia frontosa* Say, and described four new species, giving a key for the separation of the five species mentioned. He discarded the relative lengths of the aristal segments as a character variable in individuals and noted the colour of the antennae, colour of the thoracic vestiture, and length of the claws and pulvilli in the males.

Townsend (19) added another species, *Gonia sagax* Townsend.

Coquillet (3) reduced the previously described species to two, synonymizing *Gonia sagax* Townsend and *Gonia senilis* Williston, and synonymizing *Gonia frontosa* Say, *Gonia exul* Williston, and the European species *Gonia capitata* DeGeer, and described a third, new species, *Gonia turgida* Coquillet. *Gonia porca* Williston he recorded as "unrecognized".

Smith (15) described a new species, *Gonia distincta* Smith, basing his description on females only. In the following year Townsend (20) described a new, related genus, *Cnephalogonia* Townsend, with *Gonia distincta* Smith as the type species.

Strickland (17), working with cutworm outbreaks, found very different types of larvae of *Gonia* present. Tothill (18) revised the genus, largely on material reared by Strickland. Tothill's was the first classification based on male genitalia, the forceps of the genitalia being described but not figured. He recognized 17 species, 10 of which he described as new. *Gonia frontosa* Say, *Gonia sagax* Townsend, *Gonia senilis* Williston, *Gonia porca* Williston, and *Gonia sequax* Williston, he recognized as distinct species along with *Gonia distincta* Smith and *Gonia turgida* Coquillet.

Reinhard (12) described an additional new species, *Gonia texensis* Reinhard, again describing but not illustrating the male genitalia.

Present Revision of the Genus

The writer commenced work on this genus at the University of Alberta, where a considerable collection of *Gonia* had accumulated. This was augmented by specimens from the University of Montana, the Dominion Entomological Laboratory at Saskatoon, Saskatchewan, and material collected by the writer and others at Macdonald College, Province of Quebec. Dr. McDunnough, Department of Agriculture, Ottawa, kindly allowed the examination of Tothill's type material and of several hundreds of specimens in the Canadian National Collection.

Early in the investigation it became evident that, at least at present, accurate determination of female material must remain impossible. It is the hope of the writer to investigate, at a later date, the connection between the species

as here determined on male characters, and the different types of larvae (17). This, together with a study of the female genitalia, might lead to the discovery of means of classifying females.

For the purpose of the present paper, males only are considered except where the female definitely associated with these males is known. The key is based on combinations of characters of the genitalia and of other structures. The only portion of the male genitalia showing characters of taxonomic value is the anal forceps (Fig. 1). This compound structure is normally folded underneath the abdomen and concealed in the genital pouch beneath the lobes of the fifth sternite. From this position it must be extruded with a hooked needle in order that lateral and dorsal views may be observed. The terms "dorsal" and "ventral" as used in this paper refer to the forceps in this extended position. The outline of the forceps in the dorsal and lateral views offers the best characters for separating the species.

The drawings presented here were prepared from genitalia (of type materia where possible) first removed from the specimen, then treated for from 24 to 36 hr. in cold 10% potassium hydroxide, and kept in vials of 40% alcohol and 10% glycerine solution. They were examined in glycerine with the aid of a binocular microscope. A camera lucida was attached to one tube of the microscope to aid in securing the outline. Permanent mounts were made in balsam or De Faure's solution, but this is not advisable as it prevents any further moving of the material. For purposes of determination in later work, it did not prove necessary to dissect out the genitalia from the body. They were merely extruded to reveal the forceps and dried in that position. A word of warning should be given here pointing out that unless the observations are made from perfectly similar positions in all cases (i.e., lateral and dorsal views), appearances may be very misleading. The dorsal view is difficult to get in such a way that the two sides of the forceps are symmetrical. Thus an apparent asymmetry is noticeable in some of the diagrams presented in this paper.

In many species the appearance of the genitalia is not sufficiently distinctive to serve alone as a distinguishing character. Other morphological structures must then be used.

The key given has been built with one chief thought in mind; i.e., that it should be usable and make exact separation of the species possible. For this reason the species key out separately and not into the groups of related species that are discussed later.

Gonia Meigen

1803 GONIA Meigen (9). 1826 GONIA Meigen (10), (13 spp. described). 1830 RHEDIA Desvoidy (6) (Synonymy by Coquillet). 1830 REAUMURIA Desvoidy (7), (Synonymy by Coquillet). 1834 GONIA Meigen (5), (*Musca capitata* DeGeer, designated as type). 1849 GONIA Meigen (22). 1851 ISOMERYA Desvoidy (7), (Synonymy by Schiner). 1851 PISSEMYA Desvoidy (7), (Synonymy by Brauer and Bergenstam). 1862 GONIA

Meigen (14). 1878 GONIA Meigen (11). 1887 GONIA Meigen (24), (Desc. and table of spp.). 1894 GONIA Meigen (16), (Discussed generic limits). 1897 GONIA Meigen (3). 1924 GONIA Meigen (18). 1934 GONIA Meigen (21), (Greatly restricted).

Gonia Meigen is easily separated from other genera of Tachinids by the following combination of characters: head very much inflated (except in *G. distincta* Smith), yellowish except on the occiput and the eyes, pollinose with the front above largely translucent and more than twice as wide as either eye in both sexes (wider in females), the frontal vitta not strikingly darker in ground colour, ocellar bristles strong and curved backward, orbitals present in both sexes, eyes bare, antennae black or yellow, the penultimate aristal segment always more than three times as long as wide, usually a strong bend at the junction of the second and third aristal segments giving the arista a geniculate appearance, facial ridges with only a few bristles at their bases, parafacials with hair or bristles; propleura bare; lower lobes of squamae bare above; infrasquamal setulae absent. It is to be noted that the generic keys (3, 25) would eliminate *Gonia setigera* Tothill, in which the first vein is setose, from this genus. The earlier generic descriptions also list the palpi as yellow but in the *fissiforceps* group specimens show palpi with varying degrees of infuscation to dark brown. Tothill describes the first two antennal segments as yellow in all cases. This is not quite correct as frequently only a slight reddish-yellow colour on the apical portions of these segments is obvious, the segments being otherwise dark.

KEY TO THE MALES OF *Gonia* MEIGEN FOUND IN AMERICA NORTH OF MEXICO

(For the purposes of this key the genitalia are considered as extruded and extended until the forceps point backward in the direction of the long axis of the body. The terms dorsal, ventral, lateral, "turned up", and "turned down" refer to the structures in this position. Figs. 1 to 22 are lateral views of the forceps; Figs. 1a to 22a, dorsal views.)

1. First, second, and third segments of antennae entirely yellow, aristae black or mostly yellow.....2.

   At least most of the third antennal segments as well as the aristae dark in ground colour.....3.

2. Aristae mostly yellow, genal hairs white; forceps thick, straight on the ventral edge when viewed in profile, not over twice as long as the median width; as in Figs. 13, 13a.....(No. 13) *senilis* Williston.

   Aristae black, genal hairs dark; forceps three times as long as their median breadth, ventral edge convex toward the apex, as in Figs. 2, 2a.....(No. 2) *sagax* Townsend.

3. First wing vein bare with at the most one or two setae.....4.

   First wing vein setose on the dorsal side, basally, half way to the first cross vein; forceps as in Figs. 10, 10a.....(No. 10) *setigera* Tothill.

4. Forceps without a conspicuous dorsal carina.....5.
   Forceps with a strongly developed carina as deep as the forceps are thick and extending for $\frac{3}{4}$ the length of forceps....(No. 21) *carinata* Tothill.
5. Pile on the occiput white.....6.
   Pile on the occiput brown; forceps as in Figs. 3, 3a.....(No. 3) *fuscicollis* Tothill.
6. Pleurae without yellow pile; lobes of fifth sternite black; genal pile light or dark.....7.
   Pleurae with yellow pile; lobes of fifth sternite yellow; genal pile fine and yellow; forceps as in Figs. 16, 16a.....(No. 16) *porca* Williston.
7. Lobes of the fifth sternite excised laterally (as in Fig. 18b): abdomen shining black, with dorsal segmental bases narrowly or not at all pollinose.....8.
   Lobes of the fifth sternite not excised laterally; abdomen frequently reddish on the sides, dorsal segmental bases often broadly pollinose...9.
8. Forceps more than half as deep as wide at the base of the apical cleft, ventral edge almost straight, dorsal convexity extending only about one-third of the distance to the apex, which is blunt; in dorsal view with the apical cleft extending beyond the middle, as in Figs. 18, 18a.....(No. 18) *fissiforceps* Tothill.
   Forceps at the base of the apical cleft more than half as deep as wide, ventral edge almost straight but the dorsal convexity extending almost one-half the length of the forceps, apex blunt, apical cleft not extending beyond the middle, as in Figs. 20, 20a....(No. 20) *yukonensis* Tothill.
   Forceps at the base of the apical cleft not half as deep as wide, ventral edge curved ventrally at the apex, which is almost hook-like, as in Figs. 19, 19a.....(No. 19) *tenuiforceps* new species.
9. Forceps almost as deep as long, dorsally with a subapical tuft of long black setae, ventrally with a cushion of yellowish setae in the hollow between the apical lobes, as in Figs. 17, 17a..(No. 17) *texensis* Reinhard.
   Forceps much longer than deep, no cushion of yellowish setae between the apical lobes ventrally.....10.
10. Genal hairs light in colour.....11.
    Genal hairs dark in colour.....12.
11. Forceps broad as in Fig. 12a, ventral edge slightly curved dorsally at the apex as in Fig. 12, abdomen mostly yellow with a narrow dorsal dark stripe; abdominal venter yellow except at the base and apex; eastern species.....(No. 12) *sequax* Williston.
    Forceps narrow as in Fig. 9a, ventral edge straight as in Fig. 9, abdomen mostly yellow with dark dorsal stripe prominent; venter with a longitudinal black stripe.....(No. 9) *albagenae* new species

12. Parafacials narrower than the greatest width of the eye; pulvilli and ungues long as in Fig. 14b; forceps with dorsal convexity reaching almost to the apex, as in Figs. 14, 14a.....(No. 14) *distincta* Smith.

13. Parafacials unusually wide with numerous long black setae next to the eyes and directed medially, setal vestiture of front, parafacials, and genae generally denser, longer, and darker than in any other species; forceps with a straight ventral edge, dorsal convexity not conspicuous, as in Figs. 11, 11a.....(No. 11) *turgida* Coquillet.

Parafacials as usual, with scattered black setae mostly short; forceps with ventral edge curved or if straight forceps are short or have a conspicuous dorsal convexity.....14.

14. Forceps short with a straight ventral edge, with or without a strong dorsal convexity, rarely with the apex slightly dilated dorsally in *breviforceps*.....15.

Forceps short with a strongly curved ventral edge or longer with a very slightly to strongly curved ventral edge, the apex in profile always dilated dorsally, knob-like.....16.

15. Forceps short, ventral edge straight, dorsal convexity conspicuous and extending about four-fifths of the distance to the apex, as in Figs. 15, 15a; pulvilli as long as the last tarsal segment; ungues slender, as long as pulvilli, yellowish basally, as in Fig. 15b.....
(No. 15) *longipulvilli* Tothill

Forceps short, and small, ventral edge straight, apex rarely slightly dilated dorsally, as in Figs. 8, 8a; pulvilli and ungues distinctly shorter than the last tarsomere.....(No. 8) *breviforceps* Tothill.

16. Forceps 2 to 4 times as long as deep, ventral edge strongly curved, apex distinctly knob-like as in Figs. 1, 1a, small dark species; 7 mm. to 10 mm. long; sides of abdomen sometimes reddish.....
(No. 1) *frontosa* Say.

Forceps 5 to 8 times as long as deep, ventral edge almost straight to strongly curved, larger species; 9 mm. to 12 mm. long.....17.

17. Forceps in dorsal view triangular, tapering from the base to the apex, as in Figs. 4, 4a; pulvilli and ungues shorter than the last tarsomere, as in Fig. 4b.....(No. 4) *aldrichi* Tothill.

Forceps in dorsal view continuing wide from the base or expanding, then tapering rapidly near the apex, as in Figs. 5, 5a; pulvilli and ungues longer than the last tarsomere, as in Fig. 5b.....
(No. 5) *grandipulvilli* new species.

Forceps in dorsal view tapering rapidly near the base, then extending long and narrow with almost parallel sides to the apex, as in Fig. 7a..18.

18. Dorsal pile on abdomen long and erect; forceps as in Figs. 7, 7a.....
(No. 7) *discalis* new species.

Dorsal pile on abdomen short and recumbent; forceps as in *discalis* (Figs. 6, 6a).....(No. 6) *longiforceps* Tothill.
(= *brevipulvilli* Tothill).

1. *G. frontosa* Say

1829 *Gonia frontosa* Say (13). 1840 *Gonia philadelphica* Macquart (8), (Described from Philadelphia). 1849 *Gonia albifrons* Walker (22), (Described from the Hudson's Bay Territory). 1878 *Gonia frontosa* Say (11). 1887 *Gonia frontosa* Say (24), (Redescribed = *G. philadelphica* Macq., = *G. albifrons* Walk.). 1897 *Gonia capitata* (DeGeer) Coquillet (3). (Probably incorrect in synonymyzing *frontosa* with the European *capitata*) = *G. philadelphica* Macquart = *G. albifrons* Walker = *G. exul* Williston (Probably wrongly) = *G. sequax* Williston (Probably wrongly). 1905 *Gonia capitata* (DeGeer) (7) (Follows Coquillet in synonymy). 1924 *Gonia frontosa* Say, Tothill (18) (Redescribed—neotype named—no synonymy mentioned—distinguished from *G. sequax* Willst. *G. exul* Willst. not mentioned).

There seems to be little doubt of the synonymy of Say's species with *Gonia albifrons* Walker and *Gonia philadelphica* Macquart. The synonymy with *G. sequax* Williston and *G. exul* Williston suggested by Coquillet and followed by Aldrich seems improbable in view of the original description of *sequax* "with abdomen reddish yellow" and of *exul*, "with claws and pulvilli of male large." The synonymy of this species with the European *G. capitata* (DeGeer) is also doubtful. A specimen in the Canadian National Collection from Europe, and bearing the label *G. capitata* (DeGeer), determined by Bezzi, has very different forceps (Figs. 22, 22a). Williston (24), when he redescribed the species from Minnesota males, appears to have had this species, but the New England, North Carolina, and California material which he mentions was evidently a mixture of species with more red coloration on the abdomen, yellowish genal hairs, etc.

Considering the widespread distribution of this species it is probably the oldest form, phylogenetically speaking.

Type locality: upper Missouri River.

Distribution: widespread throughout Canada and United States.

Neotype: No. 785. Canadian National Collection.

2. *G. sagax* Townsend

1893 *Gonia sagax* Townsend (19). 1897 *Gonia senilis* Williston, Coquillet (3), (Synonymy probably incorrect). 1905 *Gonia senilis* Williston (1), (follows Coquillet). 1924 *Gonia sagax* Townsend (18), (Redescribed), Neotype named from Middlesex County, N.J. (Figs. 2, 2a).

The Tothill neotype of this species was captured in April, in Middlesex County, N.J. Length 10 mm., width 4 mm. Abdominal segments three and four are slightly reddish on the sides; the antennae are all yellow except the aristae which are brownish black (a feature that checks with Townsend's original description); genal hairs dark; pulvilli short, small, oval; ungues

not more than two-thirds the length of the last tarsomere; face and frontal vitta strongly yellow pollinose. Tothill has described the forceps as, "of medium length and not blunt as in *G. senilis* Williston, about three times as long as their median width and much narrower across the base of the apical cleft than at the middle."

In the Canadian National Collection is a female specimen from Aylmer, Que., 14, v, 1925, taken by G. S. Walley, with the same colour markings but very small, being barely 8 mm. long and appearing at first glance to be *frontosa* Say.

Type locality: Ames, Iowa.

Distribution: Iowa, New Jersey, Quebec (?).

Neotype: in the Canadian National Collection.

3. *G. fuscicollis* Tot.

1924 *Gonia fuscicollis* Tothill (18). (Figs. 3, 3a)

The only specimen of this species available was the male paratype, La Fayette, Ind. April, 1916 (J. M. Aldrich) in the Canadian National Collection. This specimen, Tothill tells us, bears the same data as the holotype in the United States National Museum. The general appearance of this small dark specimen (9 mm. by 4 mm.) is strikingly like *frontosa* Say as is the ventrally arcuate line of the forceps. The ventral line of the forceps is more convex than in any *frontosa* observed. The occipital pile is sparse, long, and brown.

A female from Rosthern, Saskatchewan, May 4, 1925, (K. M. King), in the Canadian National Collection, has a light brownish yellow occipital pile, but the abdomen is broadly red on the sides, the colour spreading ventrally to leave only a narrow median longitudinal dark stripe.

The occipital pile of the other species though generally white often shows varying degrees of yellowing to dark yellow, especially in specimens reared in captivity and specimens which appear to have been "wet" at some time. Further collecting may prove this species good or doubtful; at present it must be maintained on the strength of the type and paratype.

Type locality: La Fayette, Ind.

Distribution: ?

Type: United States National Museum.

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frontosa group

The three above named species have short forceps with arcuate ventral edges and appear to form a closely related group. All specimens examined have been captured in April, May, or early June.

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4. *G. aldrichi* Tothill

1924 *Gonia aldrichi* Tothill (18). (Figs. 4, 4a)

Among the many paratypes of Tothill's species *aldrichi* are two distinct species. His own description of the forceps, "unusually robust, being both long and wide. About three times as long as the median width and almost triangular in dorsal view the equal sides tapering from the base to tip. The dorsoventral flattening is very marked," is descriptive of the type, and sufficient to separate out this species. Add to this that the ungues and pulvilli in the male are distinctly shorter than the last tarsomere, and no difficulty in separation from the following species should arise.

Tothill records *aldrichi* as bred by Strickland from *Euxoa ochrogaster* and *Agrotis orthogonia* (Lepidoptera: Noctuidae). Adults are collected in April and May.

Type locality: Coaldale, Alberta.

Distribution: across Canada and the northern United States.

Type: No. 786, Canadian National Collection.

5. *G. grandipulvilli* new species (Figs. 5, 5a, 5b)

Male: length, 9 to 12 mm.; width, 4 to 5 mm. A comparatively large, dark species. Face and front light yellow with a silvery sheen in some lights, front above with a waxy appearance on the sides; lines of the face and front in profile almost straight and meeting at an angle just less than a right angle; parafacials narrower at the oral margin than at the vibrissal base, at the narrowest point wider than the greatest eye width, with an uneven double or triple row of black setae (which vary greatly in size) along the inner edge of the eye and separated from the usual row of graduated bristles along the frontal suture by a bare area. Genae less than one-half the eye height with numerous slender dark setae; antennae blackish with a greyish sheen in some lights; second antennal segment slightly reddish apically, with short dorsal setae, third segment five to seven times as long as the second; pile on the occiput white; palpi yellow. Dorsum of the thorax with five broad, longitudinal pollinose lines; scutellum yellowish at least apically, frequently dark basally, some yellow on the humeri, legs black. Pulvilli and ungues distinctly longer than the last tarsomere; the pulvilli broad and white to yellowish brown; ungues black, heavy, and extended almost at right angles to the tarsomere; tarsomeres somewhat broad and flat; wings clear with but very slight costal infuscation, first vein (R_1) bare above, third vein (R_{4+5}) with a group of setae above and below at the point where (R_{2+3}) and (R_{4+5}) separate.

Abdomen with reddish patches on the sides of segments 2 and 3 (this reddish colour though involving considerable of the abdomen in a few specimens examined is characteristically limited to separate patches of varying size on the segments mentioned). Dorsally the abdominal tergites narrowly

to broadly pollinose basally, the pollen spreading out laterally on the fifth segment to cover the sides. Dorsal vestiture short and semi-erect.

Forceps in lateral view resemble those of *aldrichi*, strong, long, and flattened; ventral edge slightly curved, apical dorsal convexity evident, dorsally with sides parallel or divaricating more than half way to the apex, then tapering rapidly to the blunt point, outline much less nearly triangular than in *aldrichi*.

Type:—Edmonton, Alta. 25/4/23. F. S. Carr, No. 5032 Canadian National Collection, (Genitalia, vial No. 27).

Paratypes:—Edmonton, Alta.: as type (vial No. 25); 2, v, 1937, F. O. Morrison; 4, v, 1937, E. H. Strickland. Lethbridge, Alta.: ? Seamans and Strickland; April 17, 1925,—April 11, 1926, 2 specimens, H. E. Gray; May 3, 1923,—April 18, 1923, H. L. Seamans. Saskatoon, Sask.: April 20, 1937, 3 specimens, A. P. Arnason; 15 iv, 1915, ?; 8, v, 1923,—April 26, 1924, 3 specimens,—22, iv, 1923,—May 11, 1925,—10, v, 1923—April 29, 1924, K. M. King; May 11, 1923,—3, v, 1923, N. J. Atkinson. Earl Grey, Sask.: 25, iv, 1926, J. D. Ritchie. Bozeman, Montana: June 2, (192?), ? (vial No. 53). Toronto, Ont.: iv, 25, 1906. Merivale, Ont.: 3, v, 1938, 2 specimens, G. E. Shewell. Maple Sap, 1, May, 1921. F'ton: 27, iv, 13. Vernon, B.C.: 28, iv, 1924, 2 specimens, E. R. Buckell. Kaslo, B.C.: 10, iv, 1907, Cockle (vial No. 35). Montreal, Que.: 17, v, 1915, J. I. Beaulne. The following are among Tothill's paratypes of *aldrichi* in the Canadian National Collection.

Ottawa: 15, April, 1915 (Genitalia slide No. 12); 1905, James Fletcher; 22, iv, 1906, 2 specimens,—1, 4, 1906, W. Metcalfe; 18, iv, 1915, A. E. Kellet; and one labelled 0.15.4. 1889. O. Ottawa. Jordan, Ont.: 20, iv, 1919, C. H. Curran. One not labelled. Mussellshell, Mont.: 5, 12, 1917, Mont. Exp. Sta. Mac. Coll.: 11/4/1915. Toronto, Can.: 16, iv, 1895, E. M. Walker; April 21, 1897? Hull, Que.: 17, April; 15 ?, 2 specimens. Logan Ut. Jn. 1915. H. R. Hogan.

6. *G. longiforceps* Tothill*

1924 *Gonia longiforceps* Tothill (18) (= *Gonia brevipulvilli* Tothill). (Figs. 6, 6a)

The figures show Tothill's description of the forceps in this species very accurately. "Forceps long and slender being about five times as long as the median width. In dorsal view the sides taper abruptly at the base and then continue almost parallel to the apex. Viewed in profile the dorsal declevity starts well beyond the middle and the ventral edge is arcuate."

* NOTE: *Names given by Tothill and ending in the word "—forceps" were published in this form. The type labels, however, carry the Latin genitive form "—forcipis". The names stand as published and the same form has been adopted for new species names involving this word.*

Where the word "forceps" itself occurs, apart from names (in the text and key) it has been used in the anglicized form and considered as plural, comparable to the word "scissors". The singular and plural Latin forms "forceps" and "forcipes" seem unnecessarily awkward.

There appears to be no difference between the type and paratypes of this species and those of *Gonia brevipulvilli* Tothill, which is here made a synonym.

Among the paratypes of this species and other material previously grouped here occur specimens that have been segregated to form the new species, *G. discalis*. This species differs from *G. longiforceps* in having the dorsal vestiture of the abdomen long, the setae often attaining a length equal to one-half the length of the segments, and borne almost erect; whereas the dorsal abdominal vestiture of *longiforceps* is short and semi-recumbent.

Gonia longiforceps is recorded as bred from *Agrotis orthogonia* (Noctuidae: Lepidoptera).

Type locality: Lethbridge, Alberta.

Distribution: across Canada and the United States.

Type: No. 784 Canadian National Collection.

7. *G. discalis* new species (Figs. 7, 7a)

Male, length 9 to 13 mm. Dark species. Face and front light yellow, with a silvery sheen in some lights; the front from above has a waxy appearance on the sides; parafacials only slightly narrower at the oral margin than at the vibrissal bases, narrowest width greater than the greatest eye width, with a double or triple row of black setae along the inner edge of the eye, others scattered or in broken rows inside these and the usual short row of heavier bristles along the lower edge of the frontal suture, these bristles graduated in length from the shortest at the top of the row to the longest outside and considerably above the oral vibrissae; genae about half the eye height with a vestiture of slender black setae; antennae blackish often with a greyish sheen in some lights; second antennal segment yellowish with short, heavy, dorsal setae; third segment five to seven times as long as second. Pile on the occiput white. Palpi yellow. Dorsum of thorax black with five indistinct longitudinal pollinose lines; scutellum and small area preceding it yellow; some yellow on humeri. Legs black. Pulvilli and ungues distinctly shorter than last tarsomere. Wings slightly infuscated basally on the dorsal edge.

Abdomen shining black, segments 3, 4, and 5 pollinose basally, red coloration occurs on segments 3 and 4 and sometimes 5 in varying amounts. The dorsal abdominal vestiture on segments 3 and 4 is long and erect or almost erect. The setae reach a length equal to half the length of these segments.

Forceps similar to those of *longiforceps*, dorsally, wide at the base, tapering rapidly then extending with almost parallel sides to the apex. In lateral view the basal dorsal convexity extends less than one-half the length of the forceps; the apex is expanded dorsally, knob-like.

Type:—Nicola, B.C.: 28, iv, 1922, P. N. Vroom, No. 5003 Canadian National Collection.

Paratypes:—Nicola, B.C.: 23, v, 1922, (vial No. 114),—29, v, 1922, P. N. Vroom. Vernon, B.C.: 13, 4, 1927,—20, iv, 1927, I. J. Ward. Agassiz, B.C.: 29, iii, 1924,—6, iv, 1922. R. Glendenning. Cranbrook, B.C.: 11, v, 1922, 3 specimens,—19, v, 1922,—10, v, 1922,—12, v, 1922,—8, v, 1922, C. B. D. Garrett. Penticton, B.C.: 12, iv, 1927, E. R. Buckell. Copper Mtn., B.C.: 8, iv, 1928. G. Stace Smith. Toronto, Ont.: 13, 4, 1905. Ottawa, Ont.: 22, iv, 1906, W. Metcalfe (Slide No. 13). Earl Grey, Sask.: 25, iv, 1926, J. D. Ritchie. Saskatoon, Sask.: 27, iv, 1923, K. M. King. Lethbridge, Alta.: April 30, 1915, E. H. Strickland.

The following paratypes of *longiforceps* Tothill are in the Canadian National Collection.

Vancouver, B.C.: 10, viii, 1907,—1, 4, 1916, R. S. Sherman. Treesbank, Man.: 17, iv, 1908, J. B. Wallis. Aweme, Man.: 16, v, 1921, N. Criddle. Chilicotin, B.C.: 24, iv, 1920, E. R. Buckell (Genitalia vial No. 115).

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aldrichi group

Gonia aldrichi, *G. grandipulvilli*, *G. longiforceps*, and *G. discalis* form a second group of species all closely related. The dark coloration and arcuate ventral edge and apical swelling of the forceps suggest a close relationship to the first or *frontosa* group. In general the forceps in this group are longer and stronger than in the first and the flies are larger. Specimens have been captured throughout the breadth of the United States and Canada during the spring months.

The third or *breviforceps* group, which follows, is also taken in the spring, but the ventral edge of the forceps is straight and the distribution of the species is largely limited to central and western Canada and the United States.

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8. *G. breviforceps* Tothill

1924 *Gonia breviforceps* Tothill, (18). (Figs. 8, 8a)

From this species as described by Tothill the following new species, *G. albagenae*, has been separated. The new species includes one paratype of *breviforceps* Tothill, Vernon, B.C., 25, v, 1919. W. B. Anderson, genitalia slide No. 14, Canadian National Collection. *G. breviforceps* has distinctly dark setae on the genae while *G. albagenae* has a vestiture of fine white setae on the genae except for a few darker hairs at the lower edge in some specimens. In general the parafacials are slightly narrower in *breviforceps* Tothill.

G. breviforceps has been bred from *Euxoa ochrogaster* both in Alberta and Saskatchewan.

Type locality: Lethbridge, Alberta.

Distribution: British Columbia, Montana, California, Colorado.

Type: No. 788, Canadian National Collection.

9. *G. albagenae* new species (Figs. 9, 9a)

Male: length 9.5 to 11.5 mm. Specimens usually show considerable red, but dark ones occur. Face and front light yellow, with a silvery sheen in some lights; front from above has a waxy appearance on the sides; parafacials narrowing very little from the aristal bases to the vibrissae, at the narrowest point about equal in width to the greatest eye width, with an uneven double or triple row of slender black setae along the inner edge of the eye and others scattered or in broken rows inside these, the usual short row of graduated bristles along the frontal suture; genae about half eye height, covered with sparse fine white setae, at the most a few dark setae occur next to the basal row of bristles; antennae blackish with a greyish sheen in some lights; second segment yellowish with short heavy dorsal setae, third segment 5 to 7 times as long as second. Pile on the occiput white. Palpi yellow. Dorsum of thorax black with five indistinct longitudinal pollinose lines; scutellum, small lateral areas preceding it, and parts of humeri yellowish. Legs black. Pulvilli and ungues distinctly shorter than last tarsomere. Wings clear with very slight costal infuscation.

Abdomen usually broadly reddish on the sides of segments 3 to 4, the coloured areas sometimes extending to other segments and ventrally, leaving only basal and apical dark rings connected by a narrow dark stripe dorsally or by dark dorsal and ventral stripes. Lobes of fifth sternite dark. Dorsal abdominal vestiture short and semi-erect.

The forceps resemble those of *breviforceps*, short and straight on the ventral edge, little dorsal convexity, dorsally with almost parallel sides for the greater part of their length.

A female (Vaisseaux, B.C., 14, vi, 1919, W. B. Anderson), apparently of the same species has similar body markings and white genal hairs.

Type:—Penticton, B.C.: 12, iv, 1927, E. R. Buckell, No. 5034 Canadian National Collection.

Paratypes:—Penticton, B.C.: 21, iv, 1927, E. R. Buckell (Genitalia vial No. 113). Lillooet, B.C.: ? A. W. A. McPhair. Naramata, B.C.: 2, v, 1919, E. R. Buckell (Genitalia vial No. 132). A paratype of *breviforceps* Tothill in the Canadian National Collection. Vernon, B.C.: 25, v, 1919. W. B. Anderson (Genitalia slide No. 14).

10. *G. setigera* Tothill

1924 *Gonia setigera* Tothill, (18). (Figs. 10, 10a)

Drawings were prepared from the one male paratype (No. 781 Canadian National Collection) with same data as the type. Two male specimens, one from Penticton, B.C., 21, iv, 1927, E. R. Buckell, with forceps exactly as in the paratype and a second from Oliver, B.C., 23, iv, 1927, (Genitalia vial No. 131), E. R. Buckell, of which the forceps show a more evident dorsal convexity but are otherwise similar, are in the Canadian National Collection. Males and females of this species are easily distinguishable by the numerous

setae on the first vein (R_1). The third vein (R_{4+5}) usually bears a greater number of setae than in any other species. As was pointed out in dealing with the generic limits of *Gonia* the generic keys of Williston and Coquillet exclude this species. In examining other species one specimen was noticed with one seta dorsally on the first vein of one wing only.

Type locality: Essex, Mass.

Distribution: Massachusetts, California, British Columbia.

Type: in the Museum of the Boston Society of Natural History.

11. *G. turgida* Coquillet

1897 *Gonia turgida* Coquillet, (3). 1924 *Gonia turgida* Coquillet, (18). 1936 *Cystogonia turgida* (Coq.) Townsend, (21). (Type of new genus *Cystogonia* Townsend). (Figs. 11, 11a).

Drawings were made from one of several male specimens from Idaho, May 2, 1919, E. H. Quales, in the Canadian National Collection, probably placed in this species by Tothill who redescribed the species from a male taken by E. G. Holt at Round Mountain, Nevada, 6,300 ft. (The writer was unable to locate this specimen.) Tothill had his specimen compared with the type by Aldrich, who mentions the striking colour pattern of the type. Among the material received from Saskatoon are two specimens, one from Three Mile Creek, Sask., 23, v, 1921, A. E. Cameron, and one labelled Mortlach, 31, v, 1909, which are evidently the same species. The original description of Coquillet, "the front near the eyes densely covered with rather long bristly hairs, sides of face each one and one-half times as wide as the median depression, densely covered with rather long black bristly hairs which are less numerous along the facial ridge," is sufficient to assure the identity of these specimens. The width of the parafacials compared to the eye width as used in Tothill's key is, alone, however, totally inadequate as a determining character.

Type locality: Los Angeles, California.

Distribution: California, Idaho, Saskatchewan.

Type: No. 3640 United States National Museum.

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breviforceps group

The writer considers the straight ventral edge and general shape of the forceps in this group to be indicative of close relationship. The body coloration is characterized by a distinct dorsal, longitudinal dark stripe on the abdomen. Frequently a ventral dark stripe is also present.

There appears to be a decided break between the three groups of species described above; i.e., the *frontosa*, *aldrichi*, and *breviforceps* groups and the two groups to follow. Adults of all species dealt with up to this point are collected in the spring months, while all those to follow are found in the late summer. This suggests a different host relationship and means of spending

the winter. No host records of the following species are known to the writer. Moreover, much wider variations in genital and other characters occur among the following forms. They are, as far as we have evidence, to date, generally more restricted in their habitats as well as specialized in genital structures.

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12. *G. sequax* Williston

1887 *Gonia sequax* Williston (24). 1897 *Gonia capitata* (DeGeer). Coquillet (3). = *Gonia frontosa* Say, = *Gonia philadelphica* Macquart, = *Gonia albifrons* Walker, = *Gonia exul* Williston (probably incorrect). 1905 *Gonia capitata* (DeGeer) (1) (follows Coquillet). 1924 *Gonia sequax* Williston (18) (redescribed). (Figs. 12, 12a).

The drawings were prepared from a male specimen from Jordan, Ont., 23, viii, 1915, C. H. Curran, in the Canadian National Collection. This locality is mentioned by Tothill, who no doubt examined this specimen. Four male and five female specimens were taken by the writer and his associates at Macdonald College during August and September, 1938. It is not clear whether Tothill examined the type material of *sequax*, but the large amount of yellow on the body stressed by Williston in his original description together with the short pulvilli make this determination fairly certain. Females taken at Macdonald College show a more widespread darkening on the dorsal surface of the abdomen than do the males which have a very narrow continuous or broken dorsal, dark stripe widening out at the base and apex of the abdomen. No ventral longitudinal dark stripe is present. The genal hairs are yellow.

Synonymy with *G. frontosa* Say can be discarded on colour, size, colour of genal hairs, and the appearance of the forceps. Synonymy with *G. capitata* (DeGeer) is similarly denied by the light colour and the yellow genal hairs. The forceps show a somewhat weaker dorsal convexity than do those of the specimen determined as *G. capitata* (DeGeer) by Bezzi (See Figs. 22, 22a) but are otherwise similar.

Tothill makes no mention of *G. exul* Williston. Coquillet and Aldrich synonymized this species with *sequax* Williston. It seems probable that the female described as *exul* was the female of *sequax*. It is not possible to be certain of this since females cannot be separated as definitely as males. However, this description fits females taken at Macdonald College along with males of *sequax*. The California males with long pulvilli mentioned by Williston and considered conspecific with the described females were most probably *G. longipulvilli* Tothill, while the North Park specimen with narrow parafacials was *G. distincta* Smith.

Type locality: California.

Distribution: California, Colorado, Texas, Connecticut, Massachusetts, British Columbia, Ontario.

Type: ?

13. *G. senilis* Williston

1887 *Gonia senilis* Williston (24). 1897 *Gonia senilis* Williston, Coquillet (3), = *Gonia sagax* Townsend (synonymy probably incorrect). 1905 *Gonia senilis* Williston (1) (follows Coquillet). 1924 *Gonia senilis* Williston, Tothill, (18), (redescribed as distinct from *sagax* Townsend). (Figs. 13, 13a).

Drawings were prepared from a specimen in the Canadian National Collection labelled, Oak Grove, Virginia, Fla., Daucus, 2, viii, ?, C. H. Townsend. This locality is mentioned by Tothill in his redescription of this species. Among other specimens examined were: one male from Severn, Ont., 16, 6, 1925, C. H. Curran; one male from College Park, Indiana, W. R. Walton; and two females apparently of the same species from Robinson, Delaware Co., Iowa, vii, 24, 1924, N. K. Bigelow. The suggested synonymy of this species with *sagax* Townsend is incorrect if the specimens available are accurately determined. The differences in the forceps, which are shorter and much broader in *senilis* Williston, are evident from the diagrams. Tothill separated the two species on the presence of dark setae on the genae in *sagax* and yellow setae in *senilis*. In the male from Severn, Ont., and one female from Delaware, at least numerous of the basal genal hairs are dark in some lights. The colour of the aristae, which in the original descriptions is given as orange yellow except at the tip in *senilis* and as brown in *sagax*, appears to be a good character but should be borne out by the forceps.

The differences in the forceps together with the fact that *sagax* has been captured in the spring months and *senilis* in the summer has led the writer to adopt the grouping in this paper and separate these two species so widely in spite of the similar colour of the third antennal segments.

Type locality: Western Kansas.

Distribution: Indiana, Virginia, New Jersey, Georgia, Florida, Ontario.

Type: ?

14. *G. distincta* Smith

1915 *Gonia distincta* Smith (15). 1916 *Gonia distincta* Smith, Townsend (20), (made the type of a new genus *Cnephalogonia* Townsend). 1924 *Gonia distincta* Smith, Tothill (18). (Figs. 14, 14a, 14b).

Gonia distincta Smith was described from three female specimens and the original description, except for the colour, gives us little information of specific value, especially as the specimens were female. Townsend, making this the type of his new genus, adds: "Female. Front not swollen . . . no median marginal macrochaetae on the first* abdominal segment; no closely set marginal machrochaetae on the third* segment. Parafacials below not over one-half greatest eye width, widening above to nearly eye width at base of antennae. Front marginal macrochaetae of parafacials sparse, few and weak."

* *"First" and "third" refer to the "second" and "fourth" segments respectively, as understood in the second part of this paper.*

Tothill redescribed the species from five males and two females, one of the females having been compared with the type described by Mr. C. W. Johnson. The narrow parafacials are mentioned and the "abdomen in males yellow with a wide black dorsal stripe that spreads out posteriorly to cover part of the third and all of the fourth tergum, and with a median ventral longitudinal black stripe also; in the females, black." The forceps are also briefly described.

Townsend (21), in his Manual of Myiology, separates his genus *Cnephalogonia*, of which this species is the type, from related genera on the absence of median marginals on the first segment. This character is purely sexual, these median marginals being present in males of this species and absent in the females of many other species.

The writer prepared his diagrams from a specimen labelled Bar Harbor, Me., 3, viii, 24 C. W. Johnson in the Canadian National Collection, one of the specimens studied by Tothill. Other specimens examined included: two males from Mt. Desert, Me.; two from Aweme, Man.; two from Blackburn, Ont.; 11 males from Low Bush, Ont.; eight females from Low Bush; two from Blackburn and one from Mt. Desert, all in the Canadian National Collection, and one male from Bozeman, Montana.

It seems of value to redescribe this species more fully or at least fill in previous descriptions.

Male: length, 9 to 12 mm. Face and front much less swollen than in any other species of *Gonia*. Parafacials much narrower at the vibrissae than at the aristal bases with two or three uneven rows of setae, which increase in size mesally, and the usual row along the frontal suture. No bare space between other setae and this last row. Genal setae dark, genae about one-third eye height. Occipital pile white. The dorsum of the thorax in certain lights appears to have the usual five wide longitudinal, pollinose lines separated by narrow dull dark lines. In other lights the narrow dull black lines appear pollinose, the wide lines shiny black. The scutellum, varying areas cephalic to the scutellum, and the humeri, yellow. Legs black. *Pulvilli and ungues longer than the last tarsal segment.* Pulvilli white. Ungues slender, curved at the tips and in strong light yellow except at the tips. The abdomen has been well described as to colour by Tothill (quoted above). In most cases it is more orange than yellow. The dorsal and ventral longitudinal stripes are constant and conspicuous; the segmental bases are broadly pollinose. The pollen spreads out especially on the sides of segments 4 to 5 almost the length of the segments. A pair of marginal macrochaetae are present on the second (Townsend's first) segment and a second pair on the third segment. The fourth bears a row of long, strong, marginals, the fifth a row of submarginals with a row of shorter bristles caudad of these.

The forceps are covered on the dorsal side with long, slender, black setae; the dorsal convexity extends down almost to the apex giving them a singular resemblance to those of *longipulvilli* Tothill; however, the extension of the convexity in the last mentioned species is not as great.

Female. Face less swollen than in male, appearing almost flattened. Parafacials wider than in male, considerably narrower at vibrissae than at aristal bases, width at vibrissae about two-thirds greatest eye width.

Thorax as in male. Pulvilli and ungues variable in individuals, from two-thirds to full length of last tarsomere; ungues slender and yellowish basally. Abdomen shining black with only obscured reddening on the sides and venter, no median marginals on the second segment.

Segments pollinose basally, pollen spreading laterally on segments 4 and 5.

Type locality: Westport Factory, Mass.

Distribution: Mass., Conn., Maine, Man., Sask., Mon., Ont.

Type: collection of Boston Society of Natural History.

The North Park specimen referred to by Williston when describing *Gonia exul* was probably of this species.

15. *G. longipulvilli* Tothill

Gonia longipulvilli Tothill (18). (Figs. 15, 15a, 15b)

The drawings were prepared from type No. 789 in the Canadian National Collection. Specimens examined have the pulvilli and ungues of the male longer than the last tarsomere; a narrow or broken dark, longitudinal dorsal line on the abdomen, the remainder of the abdomen laterally and ventrally except for the very base and the apex suffused with an orange yellow colour, no ventral longitudinal dark line being present; forceps with the dorsal convexity extending about three-quarters of their length. The California specimens mentioned by Williston in section (a) following his description of *exul* were probably of this species. This species has been bred from *Agrotis orthogonia* in Saskatchewan.

Type locality: Royal Oak, B.C.

Distribution: the central and western parts of North America from north to south.

Type: in the Canadian National Collection.

16. *G. porca* Will.

1887. *Gonia porca* Williston (24). 1924 *Gonia porca* Williston, Tothill (18), (redescribed). (Figs. 16, 16a)

Drawings were made from a specimen labelled Lillooet, B.C. Aug., 1917, one of the specimens examined by Tothill during his study. As Tothill has pointed out, the yellow hair on the pleurae and the mesonotum make identification of this species simple as do the yellow lobes of the fifth sternite and the shape of the forceps. The depth and chisel-like apex of the forceps is an extreme modification which sets this species very distinctly apart.

Several previously undetermined specimens are in the Canadian National Collection from Jesmond, B.C., 23, vii, 1932, J. K. Jacob, at altitude 7,000

to 7,500 ft., and a series from Lake Louise, Banff, Alta., 22, vii, 1938, G. S. Walley, 8,600 ft.

Type locality: Mt. Hood, Oregon.

Distribution: Oregon, British Columbia, Alberta, Colorado, North Mexico. (Mountainous regions).

Type: ?

17. *G. texensis* Reinhard

1924 *Gonia texensis* Reinhard (12). (Figs. 17, 17a)

The drawings were prepared from paratype No. 791 in the Canadian National Collection, with the same data as the type. This species has been adequately described including the forceps. These latter structures resemble those of *G. porca* Williston in their unusual depth but are greatly modified in shape and vestiture setting this species, too, distinctly apart from all others.

Reinhard says, "In relationship this species is probably nearest to *angusta* Macquart which in Aldrich's catalogue is listed as a synonym of *pallens* Wiedmann, described from Brazil."

(*Gonia angusta* Macquart was described in Diptera Exotique, Paris, Vol. II (3): 51. The author notes that the abdomen is narrower than the thorax, a condition peculiar to *texensis* in our fauna. In 1849 Walker, in Diptera in the British Museum iv: 797, records *angusta* Macq. from Jamaica.)

Type locality: College Station, Texas.

Distribution: Texas.

Type: United States National Museum, Washington.

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sequax group

The above six species form the *sequax* group. The forceps are, in general, deep. Like the group to follow, and unlike the preceding three groups, they are collected in the late summer. The last two species, at least, are widely divergent from the close relationships so evident among other species and are similarly of limited habitat. It seems probable that transition forms between these and other types may exist.

The last or *fissiforceps* group consists of three species so closely related as to cause some doubt as to their specific rank and yet so widely separated from all the other species as to stand alone. Their habitat only they have in common with *porca* Williston, and their occurrence in late summer in common with the *sequax* group.

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18. *G. fissiforceps* Tothill

1924 *Gonia fissiforceps* Tothill (18). (Figs. 18, 18a, 18b)

This large, dark, mountain species is easily recognizable from the diagrams given here and from the description by Tothill. It might be added that the

palpi vary from yellow to black, the pulvilli are white and almost as long as the last tarsomere in males. The "escalloped" inner edges of the lobes of the fifth sternite are characteristic of this and the following two species.

The new species *tenuiforceps* is being separated from this species on the shape of the forceps alone. One of Tothill's paratypes labelled Banff, Alta., N. B. Sanson, belongs to the new species.

Type locality: Lillooet, B.C.

Distribution: Banff (Alberta), Colorado, Washington, California, Ontario.

Type: No. 782, Canadian National Collection.

19. *G. tenuiforceps* new species. (Fig. 19, 19a)

Male: length, 10 to 11 mm. Shiny black species. Face and front light yellow, with a silvery sheen in some lights; the front above with a waxy appearance on the sides; parafacials not narrowing greatly from opposite aristal bases to oral margin, at the narrowest point slightly less than or equal to the greatest eye width, with a double or triple irregular row of slender black setae along the inner edge of the eye. The usual row of setae along the edge of the frontal suture irregular in extent and in size of bristles; genae about one-half the eye height with a few scattered black setae. Antennae black with a greyish tinge in some lights, second segment reddish or yellowish apically with short, dark, dorsal setae; third segment five to seven times as long as the second. Pile on the occiput white. Palpi yellow through various degrees of infuscation to black. Thorax shiny black, sometimes with powdering of white pollen, which may be distinctly divided into five lines by very narrow shiny areas; scutellum yellow; legs black, femora and tibiae (each or both) in strong light may show yellow areas. Pulvilli and ungues (about) as long as last tarsomere. Pulvilli white. Ungues slender, and, in strong light, yellow at the base and tip.

Abdomen shining black, segments 3, 4, and 5, sometimes narrowly, evenly white pollinose basally. Rarely with indications of yellowish red colouring on the sides of segments 2, 3, and 4. The lobes of the fifth sternite are definitely excised laterally on the inside, giving a "scalloped" effect (Fig. 18b), as in *fissiforceps* and *yukonensis*.

Forceps long, resembling those of *fissiforceps* but having much less depth medially, dorsal convexity extending less than one-third of their length, apex turned ventrally (forward) to form a hook, in dorsal view the median apical cleft extends beyond the middle.

Type: Male, Hopedale, Labrador, 21, vii, 1926, W. W. Perret,—No. 5035 Canadian National Collection.

Paratypes: As above; 23, vii, 1926,—17, vii, 1926,—25, vii, 1924,—10, vii, 1926,—22, vii, 1926. Two females 18, vii, 1926. One paratype of *fissiforceps* Tothill, Banff, Alta., ?, N. B. Sanson.

20. *G. yukonensis* Tothill

Gonia yukonensis Tothill (18). (Figs. 20, 20a, 20b)

Drawings were prepared from the type, No. 786, Canadian National Collection.

Tothill records a paratype of this species from Tennessee Pass, Colo. (in the United States National Museum). His description of the median apical cleft of the forceps "extending about one third distance to base" proved a little inaccurate, when the forceps had been treated in potash. The cleft is seen to extend almost half the distance to the base. The similar habitat and the shape of lobes of the fifth sternite (not mentioned by Tothill) suggest a very close relationship with *fissiforceps*. The forceps, however, have the dorsal convexity extending further down the length of these structures (about one-third the distance to the apex) than in any *fissiforceps* observed. For the present it is thought best to maintain this name but there is considerable doubt in the writer's mind as to the specific rank of this specimen.

Type locality: Yukon Territory.

Distribution: Yukon, Colorado.

Type: No. 786, Canadian National Collection.

21. *G. carinata* Tothill

1924. *Gonia carinata* Tothill (18).

There being no known specimens of this species besides the holotype, which was not available to the writer, diagrams of this species could not be included. The following is Tothill's original description:

"Parafacials at narrowest point about three times the length of the second antennal segment and only slightly wider at the base of the antennae than at vibrissae. Genal hairs fuscous. Third antennal segment black. Occipital pile white. Pleura without golden pile. First vein without bristles; third vein with a few small bristles at base. Abdomen reddish yellow except for a dorsal and a ventral narrow black stripe and except for the last tergum which is black.

"The forceps of genitalia remarkable for the long median carina that is as deep as the forceps are thick. Otherwise the forceps are long and narrow with the notch confined to the apical fourth, and straight in ventral profile."

Holotype: Male; from Salt Lake, Utah; in United States National Museum.

Tothill merely listed the species in the order in which they occurred in his key, though he suggested a western origin and eastward spread of this genus. The information at hand seems inadequate to support or deny this hypothesis. However, the tentative order of the species in this paper is based on distribution, time of occurrence, and especially similarities in the structure of the forceps.

Frontosa is our most widely spread species, *fuscicollis* is undoubtedly closely related to it, and *sagax* though unique in its antennal colour resembles these two in the structure of the forceps. In the *aldrichi* group the forceps maintain to some extent their curved ventral edge and dorsal apical convexity but have become elongated and flattened. In the *breviforceps* group the ventral edge of the forceps has become straight and an increase in depth is evident. Moreover, the range appears more restricted. The *sequax* group includes species from widely separated areas. They are separated from each other more distinctly than are the members of other groups. In all these species the forceps have increased markedly in depth. The *fissiforceps* group includes, as previously noted, extremely closely related species, but as a group shows no close affinities to any other species. The habitat of the last group is limited to mountainous areas. It may thus overlap the habitats of *porca* and possibly *distincta*. The shape of the lobes of the fifth sternite and the shape of the forceps set the *fissiforceps* group apart.

Since *carinata* could not be examined nor even the date of capture ascertained it could not be fitted into this arrangement and is consequently listed last.

Acknowledgments

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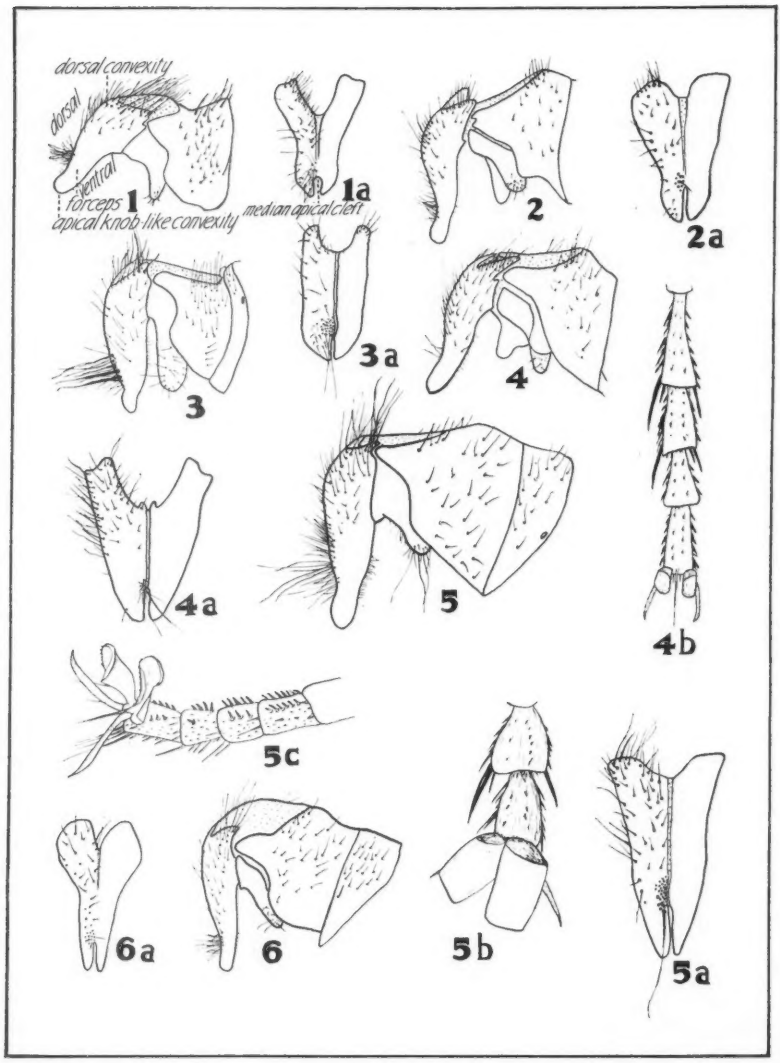
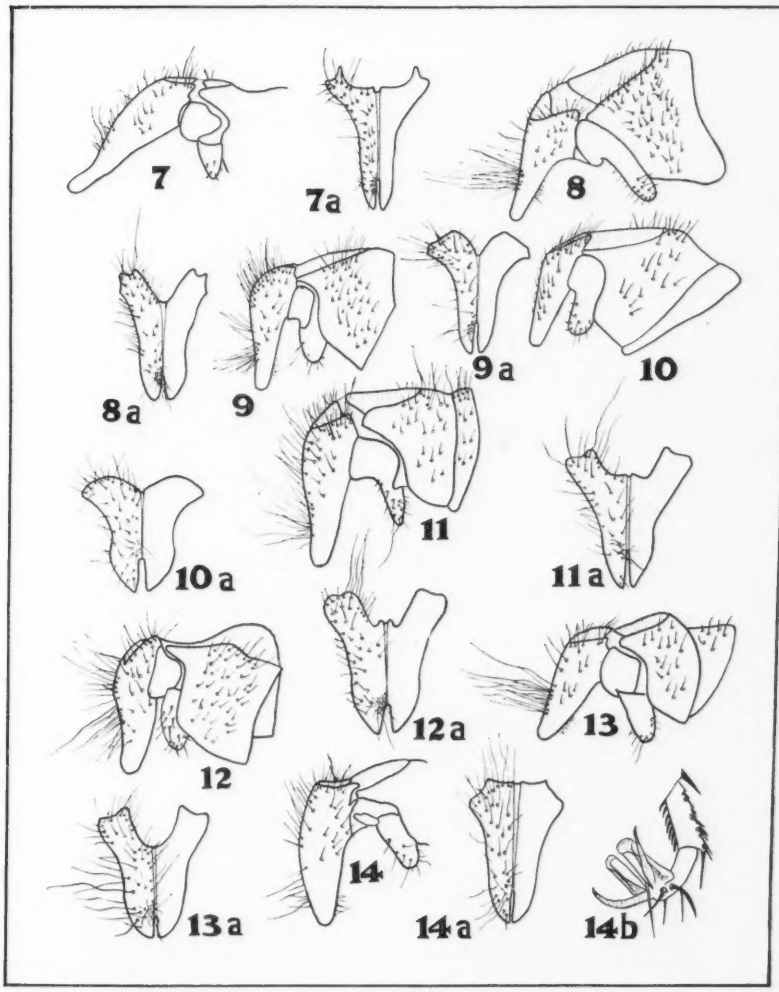
Explanation of Figures

Figs. 1 to 22 have been prepared in so far as possible from type material. In many cases the sclerites behind the forceps were broken or twisted and were drawn as observed. The exact position of the structures when drawn was that in which the forceps were most distinctive, and it varies slightly with different specimens. In all cases it is the contour of the forceps and not the exact shape of the other sclerites that is specific. The density, distribution, and length of setal vestiture varies and is often affected by the way material has been handled. It has, however, been indicated so far as possible. The linear magnification is in all cases approximately 30.



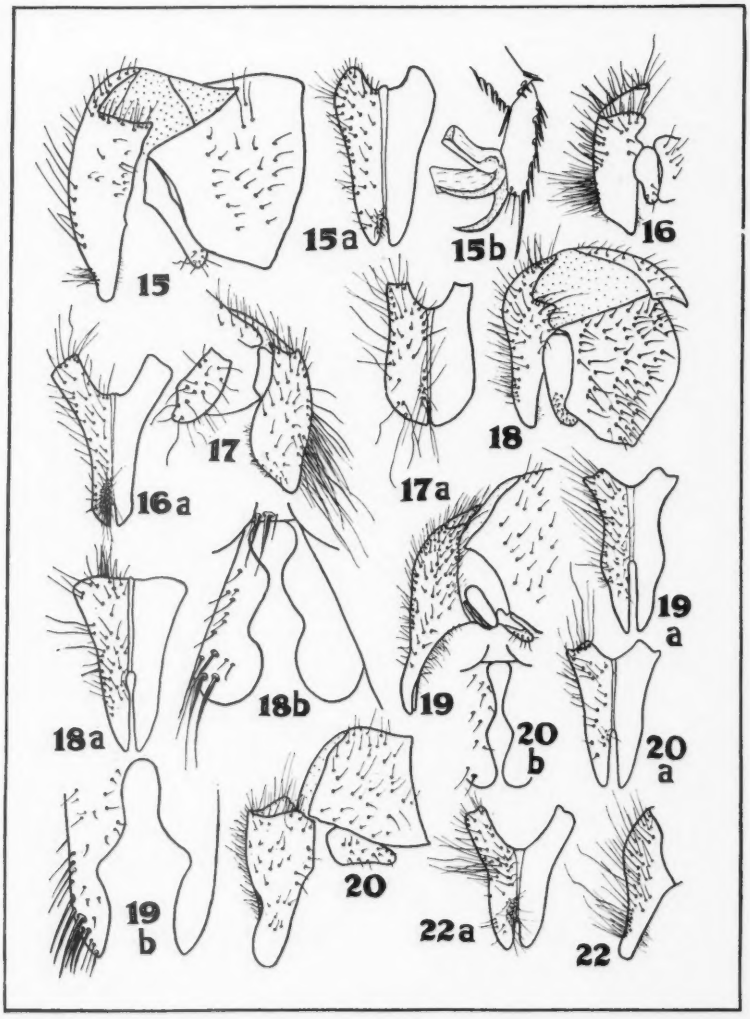
Figs. 1 to 6a.

1. *Lateral view of the forceps of G. frontosa Say.* 1a. *Dorsal view of the forceps of G. frontosa Say.* 2. *Lateral view of the forceps of G. sagax Townsend.* 2a. *Dorsal view of the forceps of G. sagax Townsend.* 3. *Lateral view of the forceps of G. fuscicollis Tothill.* 3a. *Dorsal view of the forceps of G. fuscicollis Tothill.* 4. *Lateral view of the forceps of G. aldrichi Tothill.* 4a. *Dorsal view of the forceps of G. aldrichi Tothill.* 4b. *Anterior tarsus, claws and ungues of G. aldrichi Tothill.* 5. *Lateral view of forceps of G. grandipulvilli new species.* 5a. *Dorsal view of the forceps of G. grandipulvilli new species.* 5b. *Dorsal view of last two tarsomeres, pulvilli and ungues of the prothoracic leg of G. grandipulvilli, new species.* 5c. *Lateral view of the same.* 6. *Lateral view of the forceps of G. longiforceps Tothill.* 6a. *Dorsal view of the forceps of G. longiforceps Tothill.*



Figs. 7 to 14b.

7. *Lateral view of the forceps of G. discalis new species.* 7a. *Dorsal view of the forceps of G. discalis new species.* 8. *Lateral view of the forceps of G. breviforceps Tothill.* 8a. *Dorsal view of the forceps of G. breviforceps Tothill.* 9. *Lateral view of the forceps of G. albagenae new species.* 9a. *Dorsal view of the forceps of G. albagenae new species.* 10. *Lateral view of the forceps of G. setigera Tothill.* 10a. *Dorsal view of the forceps of G. setigera Tothill.* 11. *Lateral view of the forceps of G. turgida Coquillet* 11a. *Dorsal view of the forceps of G. turgida Coquillet.* 12. *Lateral view of the forceps of G. sequax Williston.* 12a. *Dorsal view of the forceps of G. sequax Williston.* 13. *Lateral view of the forceps of G. senilis Williston.* 13a. *Dorsal view of the forceps of G. senilis Williston.* 14. *Lateral view of the forceps of G. distincta Smith.* 14a. *Dorsal view of the forceps of G. distincta Smith.* 14b. *Last tarsomere, pulvilli and ungues of the prothoracic leg of G. distincta Smith.*



Figs. 15 to 22a.

15. *Lateral view of forceps of G. longipulvilli Tothill (sclerites of segment 9 badly twisted).* 15a. *Dorsal view of forceps of G. longipulvilli Tothill.* 15b. *Last tarsomere, pulvilli and ungues of the prothoracic leg of G. longipulvilli Tothill.* 16. *Lateral view of the forceps of G. porca Williston.* 16a. *Dorsal view of the forceps of G. porca Williston.* 17. *Lateral view of the forceps of G. texensis Reinhardt (sclerites of segment 9 badly broken).* 17a. *Dorsal view of the forceps of G. texensis Reinhardt.* 18. *Lateral view of the forceps of G. fissiforceps Tothill (sclerites of segment 9 badly broken).* 18a. *Dorsal view of the forceps of G. fissiforceps Tothill.* 18b. *Lobes of the fifth sternite of G. fissiforceps Tothill.* 19. *Lateral view of the forceps of G. tenuiforceps new species.* 19a. *Dorsal view of the forceps of G. tenuiforceps new species.* 19b. *Lobes of the fifth sternite of G. tenuiforceps new species.* 20. *Lateral view of the forceps of G. yukonensis Tothill.* 20a. *Dorsal view of the forceps of G. yukonensis Tothill.* 20b. *Lobes of the fifth sternite of G. yukonensis Tothill.* 22. *Lateral view of the forceps of G. capitata (DeGeer)?* 22a. *Dorsal view of the forceps of G. capitata (DeGeer)?*

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